REQUEST FORM FOR DIVISIONAL APPLICATION UNDER 37 C.F.R. §1.60

08/819669

72192 U.S. PTO

Docket Number: **LUD-5253.5 DIV**

03/17/97

03/17/97

Anticipated Classification of this Application

Class: Subclass:

Prior Application: Serial No.:

Examiner: Art Unit:

"Express Mail" mailing label No. EH473512443US

Date of Deposit March 17, 1997

I hereby certify that this paper and/or fee is being deposited with the United States Postal Service as first class mail service under 37 CFR 1.10 on the date indicated above and is addressed to the Commissioner of Patents and Trademarks, Box Patent Application, Washington, D.C. 20231

By Annette Abdelleh (print name) Annette Abdelleh (signature)

Commissioner of Patents and Trademarks
Box **PATENT APPLICATION**
Washington, D.C. 20231

This is a Request for filing a
( ) Continuation-in-Part ( ) Continuation (**X**) Divisional Application under 37 CFR 1.60 of prior application Serial No. 08/142,368, filed on May 2, 1994 entitled TUMOR REJECTION ANTIGEN PRECURSORS, TUMOR REJECTION ANTIGENS AND USES THEREOF by inventors:

Thierry BOON — Belgium
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(City and State)
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Patrick CHOMEZ — Belgium
(Full Name of Inventor) (Citizenship)
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Catia TRAVERSARI — Italy
(Full Name of Inventor) (Citizenship)
Residence: Milan, Italy
(City and State)
Post Office Address: Sesto S. Giovanni, I-20099
Milan, Italy
(City, State and Zip Code)

The above identified prior application in which no payment of the issue fee, abandonment of, or termination of proceedings has occurred, is hereby expressly abandoned as of the filing date of this new application. Please use all the contents of the prior application file wrapper, including the drawings, as the basic papers for the new application. (Note: 37 CFR 1.60 may be used for applications where the prior application is not to be abandoned).

1. ( ) Enter all amendments in the prior application.

2. (**X**) Preliminary Amendment

The filing fee is calculated on the basis of the claims existing in the prior application as amended at 1 and 2 above.

For	Number Filed	Number Extra	Rate	Basic fee ($770.00)
Total Claims.....	6 -20		x $22.00 =	$-0-
Independent Claims.	1 -3		x $80.00 =	$-0-

( ) Multiple Dependent Claims - where applicable ($260.00).

(Reduction by 1/2 for filing by small entity. If applicable, verified statement must be attached).

TOTAL FILING FEE $ 770.00

3. (**X**) A check in the amount of **$770.00** is enclosed to cover the filing fee. In the event the enclosed check is unacceptable and/or insufficient to cover the required fee, or omitted, please charge to Account No. 06-0530.

4. ( ) A new oath or declaration is included since this application is a continuation-in-part which discloses and claims additional matter.

5. ( ) Amend the specification by inserting before the first line the sentence: This application is a ( ) continuation-in-part, ( ) continuation, (**X**) divisional, of application Serial No. 08/142,368, filed May 2, 1994, now U. S. Patent Application No._____.

6. ( ) A verified statement claiming small entity status is enclosed. (Necessary even if a statement was filed in the prior application).

7. ( ) Priority of application Serial No. filed , 19 is claimed under 35 U.S.C. §119.

8. (**X**) The prior application is assigned of record to LUDWIG INSTITUTE FOR CANCER RESEARCH, 1345 AVENUE OF THE AMERICAS, NEW YORK, NY 10105

9. ( ) A petition and extension fee has been filed to extend the term in the pending prior Application until _____. A copy of the petition for extension of time in the prior Application is attached.

10. (**X**) The power of attorney in the prior application is to: Peter F. Felfe, Reg. No. 20,297; John E. Lynch, Reg. No. 20,940; Alfred H. Hemingway, Jr., Reg. No. 26,736; Vincent M. Fazzari, Reg. No. 26,879; Norman D. Hanson, Reg. No. 30,946; F. Brice Faller, Reg. No. 29,532; Andrew L. Tiajoloff, Reg. No. 31,575; John P. Luther, Reg. No. 32,261; John A. Bauer, Reg. No. 32,554; Edward P. Kelly; and Patricia A. Pasqualini, Reg. No. 34,894 my attorneys with full power of substitution and revocation; and Walter G. Weissenberger, Reg. No. 17,344.

11. ( ) Also enclosed: Request for _____-Month Extension of Time

Address all future communications to:

Norman D. Hanson, Esq.
FELFE & LYNCH
805 Third Avenue
New York, New York 10022

It is understood that secrecy under 35 U.S.C. 122 is hereby waived to the extent that if information or access is available to any one of the applications in the file wrapper of a 37 CFR 1.62 application, be it either this application or a prior application in the same file wrapper, the Patent and Trademark Office may provide similar information or access to all the other applications in the same file wrapper.

Dated: March 17, 1997

Norman D. Hanson
Reg. No. 30,946

Attorney Docket: LUD-5253.5 DIV-JEL/NDH

UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants:	Thierry Boon-Falleur et al.	Date:	March 17, 1997
Serial No.:	To Be Assigned	Filing Date:	Herewith
Group Art Unit:	Unknown	Examiner:	Unknown

Honorable Commissioner of Patents
and Trademarks
Washington, D.C. 20231

"Express Mail" mailing label No. EH473512443US

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By Annette Abdelleh (print name) Annette Abdelleh (signature)

PRELIMINARY AMENDMENT

Prior to examination, please amend this divisional application as follows:

IN THE SPECIFICATION:

Page 1,	line 1,	after "1991" add --now U.S. Patent No. 5,342,774--;
	line 5,	change "764,364" to --764,365--;
	line 6,	after "1991" add --now abandoned--.
Page 4,	line 16,	change "Fearson" to --Fearon--.

IN THE CLAIMS:

Cancel claims 1-172 without prejudice.

Please add new claims 173-178 which follow:

173. An isolated tumor rejection antigen precursor protein, said protein being a non tum-antigen.

174. The isolated tumor rejection antigen precursor of claim 173, wherein said tumor rejection antigen precursor is a human protein.

175. The isolated tumor rejection antigen precursor of claim 174, which has an amino acid sequence encoded by SEQ ID NO: 8.

176. The isolated tumor rejection antigen precursor of claim 174, wherein said tumor rejection antigen precursor is encoded by a nucleic acid molecule, the complementary nucleotide sequence of which hybridizes to SEQ ID NO: 8, under stringent conditions.

177. The isolated tumor rejection antigen precursor of claim 176, encoded by a nucleic acid molecule which has a nucleotide sequence selected from the group consisting of SEQ ID NO: 9 through SEQ ID NO: 25.

178. The isolated tumor rejection antigen precursor of claim 177, encoded by the nucleic acid molecule having the nucleotide sequence set forth in SEQ ID NO: 11 or SEQ ID NO: 12.

REMARKS

Entry of the foregoing preliminary amendment is requested. The claims correspond to those claims included in Group V of the restriction requirement of September 27, 1995, in the parent case, which has now been allowed.

Respectfully submitted,

FELFE & LYNCH

By ____________________
Norman D. Hanson
Reg. No. 30,946

NDH:aa

Attorney Docket: LUD-5253.5 DIV-JEL/NDH

UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants:	Thierry Boon-Falleur et al.	Date:	March 17, 1997
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By Annette Abdelleh (print name) Annette Abdelleh (signature)

LETTER RE:
TRANSFER OF SEQUENCE INFORMATION

The above-referenced application contains sequence information. The sequences are identical to those filed in Serial No. 08/142,368. The CRF was mailed on January 26, 1995. It is requested that the CRF submitted on January 26, 1995 be transferred to this case. A paper copy of the sequence information is attached hereto.

The undersigned hereby attests to the absence of new matter in these submissions.

Respectfully submitted,

FELFE & LYNCH

By _____
Norman D. Hanson
Reg. No. 30,946

NDH:aa
Attachment

(1) GENERAL INFORMATION:

(i) APPLICANTS: Boon-Falleur, Thierry; Van der Bruggen, Thierry; Van den Eynde, Benoît; Van Pel, Aline; De Plaen, Etienne; Lurquin, Christophe; Chomez, Patrick; Traversari, Catia

(ii) TITLE OF INVENTION: Tumor Rejection Antigen Precursors, Tumor Rejection Antigens and Uses Thereof

(iii) NUMBER OF SEQUENCES: 26

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Felfe & Lynch
(B) STREET: 805 Third Avenue
(C) CITY: New York City
(D) STATE: New York
(F) ZIP: 10022

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Diskette, 5.25 inch, 360 kb storage
(B) COMPUTER: IBM
(C) OPERATING SYSTEM: PC-DOS
(D) SOFTWARE: Wordperfect

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: 08/142,368
(B) FILING DATE: 02-MAY-1994
(C) CLASSIFICATION: 435

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: PCT/US92/04354
(B) FILING DATE: 22-MAY-1992

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: 07/807,043
(B) FILING DATE: 12-DECEMBER-1991

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: 07/764,364
(B) FILING DATE: 23-SEPTEMBER-1991

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: 07/728,838
(b) FILING DATE: 9-JULY-1991

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: 07/705,702
(B) FILING DATE: 23-May-1991

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Hanson, Norman D.
(B) REGISTRATION NUMBER: 30,946
(C) REFERENCE/DOCKET NUMBER: LUD 5253.4-US

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (212) 688-9200
(B) TELEFAX: (212) 838-3884

(2) INFORMATION FOR SEQUENCE ID NO: 1:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 462 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: genomic DNA
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

```
ACCACAGGAG AATGAAAAGA ACCCGGGACT CCCAAAGACG CTAGATGTGT GAAGATCCTG      60
ATCACTCATT GGGTGTCTGA GTTCTGCGAT ATTCATCCCT CAGCCAATGA GCTTACTGTT     120
CTCGTGGGGG GTTTGTGAGC CTTGGGTAGG AAGTTTTGCA AGTTCCGCCT ACAGCTCTAG     180
CTTGTGAATT TGTACCCTTT CACGTAAAAA AGTAGTCCAG AGTTTACTAC ACCCTCCCTC     240
CCCCCTCCCA CCTCGTGCTG TGCTGAGTTT AGAAGTCTTC CTTATAGAAG TCTTCCGTAT     300
AGAACTCTTC CGGAGGAAGG AGGGAGGACC CCCCCCCTTT GCTCTCCCAG CATGCATTGT     360
GTCAACGCCA TTGCACTGAG CTGGTCGAAG AAGTAAGCCG CTAGCTTGCG ACTCTACTCT     420
TATCTTAACT TAGCTCGGCT TCCTGCTGGT ACCCTTTGTG CC                        462
```

(2) INFORMATION FOR SEQUENCE ID NO: 2:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 675 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: genomic DNA
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

```
ATG TCT GAT AAC AAG AAA CCA GAC AAA GCC CAC AGT GGC TCA GGT GGT       48
Met Ser Asp Asn Lys Lys Pro Asp Lys Ala His Ser Gly Ser Gly Gly
                5                  10                  15

GAC GGT GAT GGG AAT AGG TGC AAT TTA TTG CAC CGG TAC TCC CTG GAA       96
Asp Gly Asp Gly Asn Arg Cys Asn Leu Leu His Arg Tyr Ser Leu Glu
            20                  25                  30

GAA ATT CTG CCT TAT CTA GGG TGG CTG GTC TTC GCT GTT GTC ACA ACA      144
Glu Ile Leu Pro Tyr Leu Gly Trp Leu Val Phe Ala Val Val Thr Thr
        35                  40                  45

AGT TTT CTG GCG CTC CAG ATG TTC ATA GAC GCC CTT TAT GAG GAG CAG      192
Ser Phe Leu Ala Leu Gln Met Phe Ile Asp Ala Leu Tyr Glu Glu Gln
    50                  55                  60

TAT GAA AGG GAT GTG GCC TGG ATA GCC AGG CAA AGC AAG CGC ATG TCC      240
Tyr Glu Arg Asp Val Ala Trp Ile Ala Arg Gln Ser Lys Arg Met Ser
```

```
 65                  70                  75                  80
TCT GTC GAT GAG GAT GAA GAC GAT GAG GAT GAT GAG GAT GAC TAC TAC      288
Ser Val Asp Glu Asp Glu Asp Asp Glu Asp Asp Glu Asp Asp Tyr Tyr
                85                  90                  95
GAC GAC GAG GAC GAC GAC GAC GAT GCC TTC TAT GAT GAT GAG GAT GAT      336
Asp Asp Glu Asp Asp Asp Asp Asp Ala Phe Tyr Asp Asp Glu Asp Asp
            100                 105                 110
GAG GAA GAA GAA TTG GAG AAC CTG ATG GAT GAT GAA TCA GAA GAT GAG      384
Glu Glu Glu Glu Leu Glu Asn Leu Met Asp Asp Glu Ser Glu Asp Glu
        115                 120                 125
GCC GAA GAA GAG ATG AGC GTG GAA ATG GGT GCC GGA GCT GAG GAA ATG      432
Ala Glu Glu Glu Met Ser Val Glu Met Gly Ala Gly Ala Glu Glu Met
    130                 135                 140
GGT GCT GGC GCT AAC TGT GCC TGT GTT CCT GGC CAT CAT TTA AGG AAG      480
Gly Ala Gly Ala Asn Cys Ala Cys Val Pro Gly His His Leu Arg Lys
145                 150                 155                 160
AAT GAA GTG AAG TGT AGG ATG ATT TAT TTC TTC CAC GAC CCT AAT TTC      528
Asn Glu Val Lys Cys Arg Met Ile Tyr Phe Phe His Asp Pro Asn Phe
                165                 170                 175
CTG GTG TCT ATA CCA GTG AAC CCT AAG GAA CAA ATG GAG TGT AGG TGT      576
Leu Val Ser Ile Pro Val Asn Pro Lys Glu Gln Met Glu Cys Arg Cys
            180                 185                 190
GAA AAT GCT GAT GAA GAG GTT GCA ATG GAA GAG GAA GAA GAA GAA GAG      624
Glu Asn Ala Asp Glu Glu Val Ala Met Glu Glu Glu Glu Glu Glu Glu
        195                 200                 210
GAG GAG GAG GAG GAA GAG GAA ATG GGA AAC CCG GAT GGC TTC TCA CCT      672
Glu Glu Glu Glu Glu Glu Glu Met Gly Asn Pro Asp Gly Phe Ser Pro
220                 225                 230                 235

TAG                                                                  675
```

(2) INFORMATION FOR SEQUENCE ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 228 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

```
GCATGCAGTT GCAAAGCCCA GAAGAAAGAA ATGGACAGCG GAAGAAGTGG TTGTTTTTTT      60
TTCCCCTTCA TTAATTTTCT AGTTTTTAGT AATCCAGAAA ATTTGATTTT GTTCTAAAGT     120
TCATTATGCA AAGATGTCAC CAACAGACTT CTGACTGCAT GGTGAACTTT CATATGATAC     180
ATAGGATTAC ACTTGTACCT GTTAAAAATA AAAGTTTGAC TTGCATAC                  228
```

(2) INFORMATION FOR SEQUENCE ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1365 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

ACCACAGGAG AATGAAAAGA ACCCGGGACT CCCAAAGACG CTAGATGTGT 50
GAAGATCCTG ATCACTCATT GGGTGTCTGA GTTCTGCGAT ATTCATCCCT 100
CAGCCAATGA GCTTACTGTT CTCGTGGGGG GTTTGTGAGC CTTGGGTAGG 150
AAGTTTTGCA AGTTCCGCCT ACAGCTCTAG CTTGTGAATT TGTACCCTTT 200
CACGTAAAAA AGTAGTCCAG AGTTTACTAC ACCCTCCCTC CCCCCTCCCA 250
CCTCGTGCTG TGCTGAGTTT AGAAGTCTTC CTTATAGAAG TCTTCCGTAT 300
AGAACTCTTC CGGAGGAAGG AGGGAGGACC CCCCCCCTTT GCTCTCCCAG 350
CATGCATTGT GTCAACGCCA TTGCACTGAG CTGGTCGAAG AAGTAAGCCG 400
CTAGCTTGCG ACTCTACTCT TATCTTAACT TAGCTCGGCT TCCTGCTGGT 450
ACCCTTTGTG CC 462
ATG TCT GAT AAC AAG AAA CCA GAC AAA GCC CAC AGT GGC TCA 504
GGT GGT GAC GGT GAT GGG AAT AGG TGC AAT TTA TTG CAC CGG 546
TAC TCC CTG GAA GAA ATT CTG CCT TAT CTA GGG TGG CTG GTC 588
TTC GCT GTT GTC ACA ACA AGT TTT CTG GCG CTC CAG ATG TTC 630
ATA GAC GCC CTT TAT GAG GAG CAG TAT GAA AGG GAT GTG GCC 672
TGG ATA GCC AGG CAA AGC AAG CGC ATG TCC TCT GTC GAT GAG 714
GAT GAA GAC GAT GAG GAT GAT GAG GAT GAC TAC TAC GAC GAC 756
GAG GAC GAC GAC GAC GAT GCC TTC TAT GAT GAT GAG GAT GAT 798
GAG GAA GAA GAA TTG GAG AAC CTG ATG GAT GAT GAA TCA GAA 840
GAT GAG GCC GAA GAA GAG ATG AGC GTG GAA ATG GGT GCC GGA 882
GCT GAG GAA ATG GGT GCT GGC GCT AAC TGT GCC TGT GTT CCT 924
GGC CAT CAT TTA AGG AAG AAT GAA GTG AAG TGT AGG ATG ATT 966
TAT TTC TTC CAC GAC CCT AAT TTC CTG GTG TCT ATA CCA GTG 1008
AAC CCT AAG GAA CAA ATG GAG TGT AGG TGT GAA AAT GCT GAT 1050
GAA GAG GTT GCA ATG GAA GAG GAA GAA GAA GAA GAG GAG GAG 1092
GAG GAG GAA GAG GAA ATG GGA AAC CCG GAT GGC TTC TCA CCT 1134
TAG 1137
GCATGCAGTT GCAAAGCCCA GAAGAAAGAA ATGGACAGCG GAAGAAGTGG 1187
TTGTTTTTTT TTCCCCTTCA TTAATTTTCT AGTTTTTAGT AATCCAGAAA 1237
ATTTGATTTT GTTCTAAAGT TCATTATGCA AAGATGTCAC CAACAGACTT 1287
CTGACTGCAT GGTGAACTTT CATATGATAC ATAGGATTAC ACTTGTACCT 1337
GTTAAAAATA AAAGTTTGAC TTGCATAC 1365

(2) INFORMATION FOR SEQUENCE ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 4698 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

```
ACCACAGGAG AATGAAAAGA ACCCGGGACT CCCAAAGACG CTAGATGTGT    50
GAAGATCCTG ATCACTCATT GGGTGTCTGA GTTCTGCGAT ATTCATCCCT   100
CAGCCAATGA GCTTACTGTT CTCGTGGGGG GTTTGTGAGC CTTGGGTAGG   150
AAGTTTTGCA AGTTCCGCCT ACAGCTCTAG CTTGTGAATT TGTACCCTTT   200
CACGTAAAAA AGTAGTCCAG AGTTTACTAC ACCCTCCCTC CCCCCTCCCA   250
CCTCGTGCTG TGCTGAGTTT AGAAGTCTTC CTTATAGAAG TCTTCCGTAT   300
AGAACTCTTC CGGAGGAAGG AGGGAGGACC CCCCCCCTTT GCTCTCCCAG   350
CATGCATTGT GTCAACGCCA TTGCACTGAG CTGGTCGAAG AAGTAAGCCG   400
CTAGCTTGCG ACTCTACTCT TATCTTAACT TAGCTCGGCT TCCTGCTGGT   450
ACCCTTTGTG CC                                            462
ATG TCT GAT AAC AAG AAA CCA GAC AAA GCC CAC AGT GGC TCA   504
GGT GGT GAC GGT GAT GGG AAT AGG TGC AAT TTA TTG CAC CGG   546
TAC TCC CTG GAA GAA ATT CTG CCT TAT CTA GGG TGG CTG GTC   588
TTC GCT GTT GTC ACA ACA AGT TTT CTG GCG CTC CAG ATG TTC   630
ATA GAC GCC CTT TAT GAG GAG CAG TAT GAA AGG GAT GTG GCC   672
TGG ATA GCC AGG CAA AGC AAG CGC ATG TCC TCT GTC GAT GAG   714
GAT GAA GAC GAT GAG GAT GAT GAG GAT GAC TAC TAC GAC GAC   756
GAG GAC GAC GAC GAC GAT GCC TTC TAT GAT GAT GAG GAT GAT   798
GAG GAA GAA GAA TTG GAG AAC CTG ATG GAT GAT GAA TCA GAA   840
GAT GAG GCC GAA GAA GAG ATG AGC GTG GAA ATG GGT GCC GGA   882
GCT GAG GAA ATG GGT GCT GGC GCT AAC TGT GCC T             916
GTGAGTAACC CGTGGTCTTT ACTCTAGATT CAGGTGGGGT GCATTCTTTA   966
CTCTTGCCCA CATCTGTAGT AAAGACCACA TTTTGGTTGG GGGTCATTGC  1016
TGGAGCCATT CCTGGCTCTC CTGTCCACGC CTATCCCCGC TCCTCCCATC  1066
CCCCACTCCT TGCTCCGCTC TCTTTCCTTT TCCCACCTTG CCTCTGGAGC  1116
TTCAGTCCAT CCTGCTCTGC TCCCTTTCCC CTTTGCTCTC CTTGCTCCCC  1166
TCCCCCTCGG CTCAACTTTT CGTGCCTTCT GCTCTCTGAT CCCCACCCTC  1216
TTCAGGCTTC CCCATTTGCT CCTCTCCCGA AACCCTCCCC TTCCTGTTCC  1266
CCTTTTCGCG CCTTTTCTTT CCTGCTCCCC TCCCCCTCCC TATTTACCTT  1316
TCACCAGCTT TGCTCTCCCT GCTCCCCTCC CCCTTTTGCA CCTTTTCTTT  1366
TCCTGCTCCC CTCCCCCTCC CCTCCCTGTT TACCCTTCAC CGCTTTTCCT  1416
CTACCTGCTT CCCTCCCCCT TGCTGCTCCC TCCCTATTTG CATTTTCGGG  1466
TGCTCCTCCC TCCCCCTCCC CCTCCCTCCC TATTTGCATT TTCGGGTGCT  1516
CCTCCCTCCC CCTCCCCAGG CCTTTTTTTT TTTTTTTTTT TTTTTTTTTT  1566
TTGGTTTTTC GAGACAGGGT TTCTCTTTGT ATCCCTGGCT GTCCTGGCAC  1616
TCACTCTGTA GACCAGGCTG GCCTCAAACT CAGAAATCTG CCTGCCTCTG  1666
CCTCCCAAAT GCTGGGATTA AAGGCTTGCA CCAGGACTGC CCCAGTGCAG  1716
GCCTTTCTTT TTTCTCCTCT CTGGTCTCCC TAATCCCTTT TCTGCATGTT  1766
AACTCCCCTT TTGGCACCTT TCCTTTACAG GACCCCCTCC CCCTCCCTGT  1816
TTCCCTTCCG GCACCCTTCC TAGCCCTGCT CTGTTCCCTC TCCCTGCTCC  1866
CCTCCCCCTC TTTGCTCGAC TTTTAGCAGC CTTACCTCTC CCTGCTTTCT  1916
GCCCCGTTCC CCTTTTTTGT GCCTTTCCTC CTGGCTCCCC TCCACCTTCC  1966
AGCTCACCTT TTTGTTTGTT TGGTTGTTTG GTTGTTTGGT TTGCTTTTTT  2016
TTTTTTTTTT GCACCTTGTT TTCCAAGATC CCCCTCCCCC TCCGGCTTCC  2066
CCTCTGTGTG CCTTTCCTGT TCCCTCCCCC TCGCTGGCTC CCCCTCCCTT  2116
```

TCTGCCTTTC CTGTCCCTGC TCCCTTCTCT GCTAACCTTT TAATGCCTTT 2166
CTTTTCTAGA CTCCCCCCTC CAGGCTTGCT GTTTGCTTCT GTGCACTTTT 2216
CCTGACCCTG CTCCCCTTCC CCTCCCAGCT CCCCCCTCTT TTCCCACCTC 2266
CCTTTCTCCA GCCTGTCACC CCTCCTTCTC TCCTCTCTGT TTCTCCCACT 2316
TCCTGCTTCC TTTACCCCTT CCCTCTCCCT ACTCTCCTCC CTGCCTGCTG 2366
GACTTCCTCT CCAGCCGCCC AGTTCCCTGC AGTCCTGGAG TCTTTCCTGC 2416
CTCTCTGTCC ATCACTTCCC CCTAGTTTCA CTTCCCTTTC ACTCTCCCCT 2466
ATGTGTCTCT CTTCCTATCT ATCCCTTCCT TTCTGTCCCC TCTCCTCTGT 2516
CCATCACCTC TCTCCTCCCT TCCCTTTCCT CTCTCTTCCA TTTTCTTCCA 2566
CCTGCTTCTT TACCCTGCCT CTCCCATTGC CCTCTTACCT TTATGCCCAT 2616
TCCATGTCCC CTCTCAATTC CCTGTCCCAT TGTGCTCCCT CACATCTTCC 2666
ATTTCCCTCT TTCTCCCTTA GCCTCTTCTT CCTCTTCTCT TGTATCTCCC 2716
TTCCCTTTGC TTCTCCCTCC TCCTTTCCCC TTCCCCTATG CCCTCTACTC 2766
TACTTGATCT TCTCTCCTCT CCACATACCC TTTTTCCTTT CCACCCTGCC 2816
CTTTGTCCCC AGACCCTACA GTATCCTGTG CACAGGAAGT GGGAGGTGCC 2866
ATCAACAACA AGGAGGCAAG AAACAGAGCA AAATCCCAAA ATCAGCAGGA 2916
AAGGCTGGAT GAAAATAAGG CCAGGTTCTG AGGACAGCTG GAATCTAGCC 2966
AAGTGGCTCC TATAACCCTA AGTACCAAGG GAGAAAGTGA TGGTGAAGTT 3016
CTTGATCCTT GCTGCTTCTT TTACATATGT TGGCACATCT TTCTCAAATG 3066
CAGGCCATGC TCCATGCTTG GCGCTTGCTC AGCGTGGTTA AGTAATGGGA 3116
GAATCTGAAA ACTAGGGGCC AGTGGTTTGT TTTGGGGACA AATTAGCACG 3166
TAGTGATATT TCCCCCTAAA AATTATAACA AACAGATTCA TGATTTGAGA 3216
TCCTTCTACA GGTGAGAAGT GGAAAAATTG TCACTATGAA GTTCTTTTTA 3266
GGCTAAAGAT ACTTGGAACC ATAGAAGCGT TGTTAAAATA CTGCTTTCTT 3316
TTGCTAAAAT ATTCTTTCTC ACATATTCAT ATTCTCCAG 3355
GT GTT CCT GGC CAT CAT TTA AGG AAG AAT GAA GTG AAG TGT 3396
AGG ATG ATT TAT TTC TTC CAC GAC CCT AAT TTC CTG GTG TCT 3438
ATA CCA GTG AAC CCT AAG GAA CAA ATG GAG TGT AGG TGT GAA 3480
AAT GCT GAT GAA GAG GTT GCA ATG GAA GAG GAA GAA GAA GAA 3522
GAG GAG GAG GAG GAG GAA GAG GAA ATG GGA AAC CCG GAT GGC 3564
TTC TCA CCT TAG 3576
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GCTAAGAGCA TCTTTTTAAA AAATATTATT GGTAAACTAA ACAATTGTTA 3676
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(2) INFORMATION FOR SEQUENCE ID NO: 6:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

Leu Pro Tyr Leu Gly Trp Leu Val Phe
5

(2) INFORMATION FOR SEQUENCE ID NO: 7:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2419 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: genomic DNA
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

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(2) INFORMATION FOR SEQUENCE ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 5674 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

(ix) FEATURE:

(A) NAME/KEY: MAGE-1 gene

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

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CGGTCTGAGG GGCGGCTTGA GATCGGTGGA GGGAAGCGGG CCCAGCTCTG       250
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(2) INFORMATION FOR SEQUENCE ID NO: 9:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4157 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: genomic DNA
(ix) FEATURE:
(A) NAME/KEY: MAGE-2 gene
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

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GTCTCAGCAC ATGTTGCAGC CAGGGCCAGT GGGAGGGGGT CTGGGCCAGT 3592
GCACCTTCCA GGGCCCCATC CATTAGCTTC CACTGCCTCG TGTGATATGA 3642
GGCCCATTCC TGCCTCTTTG AAGAGAGCAG TCAGCATTCT TAGCAGTGAG 3692
TTTCTGTTCT GTTGGATGAC TTTGAGATTT ATCTTTCTTT CCTGTTGGAA 3742
TTGTTCAAAT GTTCCTTTTA ACAAATGGTT GGATGAACTT CAGCATCCAA 3792
GTTTATGAAT GACAGTAGTC ACACATAGTG CTGTTTATAT AGTTTAGGGG 3842
TAAGAGTCCT GTTTTTTATT CAGATTGGGA AATCCATTCC ATTTTGTGAG 3892
TTGTCACATA ATAACAGCAG TGGAATATGT ATTTGCCTAT ATTGTGAACG 3942
AATTAGCAGT AAAATACATG ATACAAGGAA CTCAAAAGAT AGTTAATTCT 3992
TGCCTTATAC CTCAGTCTAT TATGTAAAAT TAAAAATATG TGTATGTTTT 4042
TGCTTCTTTG AGAATGCAAA AGAAATTAAA TCTGAATAAA TTCTTCCTGT 4092
TCACTGGCTC ATTTCTTTAC CATTCACTCA GCATCTGCTC TGTGGAAGGC 4142
CCTGGTAGTA GTGGG 4157

(2) INFORMATION FOR SEQUENCE ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 662 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

(ix) FEATURE:

(A) NAME/KEY: MAGE-21 gene

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

```
GGATCCCCAT GGATCCAGGA AGAATCCAGT TCCACCCCTG CTGTGAACCC      50
AGGGAAGTCA CGGGGCCGGA TGTGACGCCA CTGACTTGCG CGTTGGAGGT     100
CAGAGAACAG CGAGATTCTC GCCCTGAGCA ACGGCCTGAC GTCGGCGGAG     150
GGAAGCAGGC GCAGGCTCCG TGAGGAGGCA AGGTAAGATG CCGAGGGAGG     200
ACTGAGGCGG GCCTCACCCC AGACAGAGGG CCCCCAATAA TCCAGCGCTG     250
CCTCTGCTGC CAGGCCTGGA CCACCCTGCA GGGGAAGACT TCTCAGGCTC     300
AGTCGCCACC ACCTCACCCC GCCACCCCCC GCCGCTTTAA CCGCAGGGAA     350
CTCTGGTGTA AGAGCTTTGT GTGACCAGGG CAGGGCTGGT TAGAAGTGCT     400
CAGGGCCCAG ACTCAGCCAG GAATCAAGGT CAGGACCCCA AGAGGGGACT     450
GAGGGTAACC CCCCCGCACC CCCACCACCA TTCCCATCCC CCAACACCAA     500
CCCCACCCCC ATCCCCCAAC ACCAAACCCA CCACCATCGC TCAAACATCA     550
ACGGCACCCC CAAACCCCGA TTCCCATCCC CACCCATCCT GGCAGAATCG     600
GAGCTTTGCC CCTGCAATCA ACCCACGGAA GCTCCGGGAA TGGCGGCCAA     650
GCACGCGGAT CC                                              662
```

(2) INFORMATION FOR SEQUENCE ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1640 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(ix) FEATURE:

(A) NAME/KEY: cDNA MAGE-3

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

```
GCCGCGAGGG AAGCCGGCCC AGGCTCGGTG AGGAGGCAAG GTTCTGAGGG      50
GACAGGCTGA CCTGGAGGAC CAGAGGCCCC CGGAGGAGCA CTGAAGGAGA     100
AGATCTGCCA GTGGGTCTCC ATTGCCCAGC TCCTGCCCAC ACTCCCGCCT     150
GTTGCCCTGA CCAGAGTCAT C                                    171
ATG CCT CTT GAG CAG AGG AGT CAG CAC TGC AAG CCT GAA GAA    213
GGC CTT GAG GCC CGA GGA GAG GCC CTG GGC CTG GTG GGT GCG    255
CAG GCT CCT GCT ACT GAG GAG CAG GAG GCT GCC TCC TCC TCT    297
TCT ACT CTA GTT GAA GTC ACC CTG GGG GAG GTG CCT GCT GCC    339
GAG TCA CCA GAT CCT CCC CAG AGT CCT CAG GGA GCC TCC AGC    381
CTC CCC ACT ACC ATG AAC TAC CCT CTC TGG AGC CAA TCC TAT    423
GAG GAC TCC AGC AAC CAA GAA GAG GAG GGG CCA AGC ACC TTC    465
CCT GAC CTG GAG TCC GAG TTC CAA GCA GCA CTC AGT AGG AAG    507
```

GTG GCC GAG TTG GTT CAT TTT CTG CTC CTC AAG TAT CGA GCC 549
AGG GAG CCG GTC ACA AAG GCA GAA ATG CTG GGG AGT GTC GTC 591
GGA AAT TGG CAG TAT TTC TTT CCT GTG ATC TTC AGC AAA GCT 633
TCC AGT TCC TTG CAG CTG GTC TTT GGC ATC GAG CTG ATG GAA 675
GTG GAC CCC ATC GGC CAC TTG TAC ATC TTT GCC ACC TGC CTG 717
GGC CTC TCC TAC GAT GGC CTG CTG GGT GAC AAT CAG ATC ATG 759
CCC AAG GCA GGC CTC CTG ATA ATC GTC CTG GCC ATA ATC GCA 801
AGA GAG GGC GAC TGT GCC CCT GAG GAG AAA ATC TGG GAG GAG 843
CTG AGT GTG TTA GAG GTG TTT GAG GGG AGG GAA GAC AGT ATG 885
TTG GGG GAT CCC AAG AAG CTG CTC ACC CAA CAT TTC GTG CAG 927
GAA AAC TAC CTG GAG TAC CGG CAG GTC CCC GGC AGT GAT CCT 969
GCA TGT TAT GAA TTC CTG TGG GGT CCA AGG GCC CTC GTT GAA 1011
ACC AGC TAT GTG AAA GTC CTG CAC CAT ATG GTA AAG ATC AGT 1053
GGA GGA CCT CAC ATT TCC TAC CCA CCC CTG CAT GAG TGG GTT 1095
TTG AGA GAG GGG GAA GAG TGA 1116
GTCTGAGCAC GAGTTGCAGC CAGGGCCAGT GGGAGGGGGT CTGGGCCAGT 1166
GCACCTTCCG GGGCCGCATC CCTTAGTTTC CACTGCCTCC TGTGACGTGA 1216
GGCCCATTCT TCACTCTTTG AAGCGAGCAG TCAGCATTCT TAGTAGTGGG 1266
TTTCTGTTCT GTTGGATGAC TTTGAGATTA TTCTTTGTTT CCTGTTGGAG 1316
TTGTTCAAAT GTTCCTTTTA ACGGATGGTT GAATGAGCGT CAGCATCCAG 1366
GTTTATGAAT GACAGTAGTC ACACATAGTG CTGTTTATAT AGTTTAGGAG 1416
TAAGAGTCTT GttTTTTACT CAAATTgGGA AATCCATTCC ATTTTGTGAA 1466
TTGTGACATA ATAATAGCAG TGGTAAAAGT ATTTGCTTAA AATTGTGAGC 1516
GAATTAGCAA TAACATACAT GAGATAACTC AAGAAATCAA AAGATAGTTG 1566
ATTCTTGCCT TGTACCTCAA TCTATTCTGT AAAATTAAAC AAATATGCAA 1616
ACCAGGATTT CCTTGACTTC TTTG 1640

(2) INFORMATION FOR SEQUENCE ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 943 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

(ix) FEATURE:

(A) NAME/KEY: MAGE-31 gene

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

GGATCCTCCA CCCCAGTAGA GTGGGGACCT CACAGAGTCT GGCCAACCCT 50
CCTGACAGTT CTGGGAATCC GTGGCTGCGT TTGCTGTCTG CACATTGGGG 100
GCCCGTGGAT TCCTCTCCCA GGAATCAGGA GCTCCAGGAA CAAGGCAGTG 150
AGGACTTGGT CTGAGGCAGT GTCCTCAGGT CACAGAGTAG AGGGGgCTCA 200
GATAGTGCCA ACGGTGAAGG TTTGCCTTGG ATTCAAACCA AGGGCCCCAC 250
CTGCCCCAGA ACACATGGAC TCCAGAGCGC CTGGCCTCAC CCTCAATACT 300
TTCAGTCCTG CAGCCTCAGC ATGCGCTGGC CGGATGTACC CTGAGGTGCC 350
CTCTCACTTC CTCCTTCAGG TTCTGAGGGG ACAGGCTGAC CTGGAGGACC 400
AGAGGCCCCC GGAGGAGCAC TGAAGGAGAA GATCTGTAAG TAAGCCTTTG 450
TTAGAGCCTC CAAGGTTCCA TTCAGTACTC AGCTGAGGTC TCTCACATGC 500
TCCCTCTCTC CCCAGGCCAG TGGGTCTCCA TTGCCCAGCT CCTGCCCACA 550
CTCCCGCCTG TTGCCCTGAC CAGAGTCATC 580

ATG CCT CTT GAG CAG AGG AGT CAG CAC TGC AAG CCT GAA GAA 622
GGC CTT GAG GCC CGA GGA GAg GCC CTG GGC CTG GTG GGT GCG 664
CAG GCT CCT GCT ACT GAG GAG CAG GAG GCT GCC TCC TCC TCT 706
TCT AGT GTA GTT GAA GTC ACC CTG GGG GAG GTG CCT GCT GCC 748
GAG TCA CCA GAT CCT CCC CAG AGT CCT CAG GGA GCC TCC AGC 790
CTC CCC ACT ACC ATG AAC TAC CCT CTC TGG AGC CAA TCC TAT 832
GAG GAC TCC AGC AAC CAA GAA GAG GAG GGG CCA AGC ACC TTC 874
CCT GAC CTG GAG TCT GAG TTC CAA GCA GCA CTC AGT AGG AAG 916
GTG GCC AAG TTG GTT CAT TTT CTG CTC 943

(2) INFORMATION FOR SEQUENCE ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 2531 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

(ix) FEATURE:

(A) NAME/KEY: MAGE-4 gene

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

GGATCCAGGC CCTGCCTGGA GAAATGTGAG GGCCCTGAGT GAACACAGTG 50
GGGATCATCC ACTCCATGAG AGTGGGGACC TCACAGAGTC CAGCCTACCC 100
TCTTGATGGC ACTGAGGGAC CGGGGCTGTG CTTACAGTCT GCACCCTAAG 150
GGCCCATGGA TTCCTCTCCT AGGAGCTCCA GGAACAAGGC AGTGAGGCCT 200
TGGTCTGAGA CAGTGTCCTC AGGTTACAGA GCAGAGGATG CACAGGCTGT 250
GCCAGCAGTG AATGTTTGCC CTGAATGCAC ACCAAGGGCC CCACCTGCCA 300
CAAGACACAT AGGACTCCAA AGAGTCTGGC CTCACCTCCC TACCATCAAT 350
CCTGCAGAAT CGACCTCTGC TGGCCGGCTA TACCCTGAGG TGCTCTCTCA 400
CTTCCTCCTT CAGGTTCTGA GCAGACAGGC CAACCGGAGA CAGGATTCCC 450
TGGAGGCCAC AGAGGAGCAC CAAGGAGAAG ATCTGTAAGT AAGCCTTTGT 500
TAGAGCCTCT AAGATTTGGT TCTCAGCTGA GGTCTCTCAC ATGCTCCCTC 550
TCTCCGTAGG CCTGTGGGTC CCCATTGCCC AGCTTTTGCC TGCACTCTTG 600
CCTGCTGCCC TGACCAGAGT CATC 624
ATG TCT TCT GAG CAG AAG AGT CAG CAC TGC AAG CCT GAG GAA 666
GGC GTT GAG GCC CAA GAA GAG GCC CTG GGC CTG GTG GGT GCA 708
CAG GCT CCT ACT ACT GAG GAG CAG GAG GCT GCT GTC TCC TCC 750
TCC TCT CCT CTG GTC CCT GGC ACC CTG GAG GAA GTG CCT GCT 792
GCT GAG TCA GCA GGT CCT CCC CAG AGT CCT CAG GGA GCC TCT 834
GCC TTA CCC ACT ACC ATC AGC TTC ACT TGC TGG AGG CAA CCC 876
AAT GAG GGT TCC AGC AGC CAA GAA GAG GAG GGG CCA AGC ACC 918
TCG CCT GAC GCA GAG TCC TTG TTC CGA GAA GCA CTC AGT AAC 960
AAG GTG GAT GAG TTG GCT CAT TTT CTG CTC CGC AAG TAT CGA 1002
GCC AAG GAG CTG GTC ACA AAG GCA GAA ATG CTG GAG AGA GTC 1044
ATC AAA AAT TAC AAG CGC TGC TTT CCT GTG ATC TTC GGC AAA 1086
GCC TCC GAG TCC CTG AAG ATG ATC TTT GGC ATT GAC GTG AAG 1128
GAA GTG GAC CCC GCC AGC AAC ACC TAC ACC CTT GTC ACC TGC 1170
CTG GGC CTT TCC TAT GAT GGC CTG CTG GGT AAT AAT CAG ATC 1212
TTT CCC AAG ACA GGC CTT CTG ATA ATC GTC CTG GGC ACA ATT 1254
GCA ATG GAG GGC GAC AGC GCC TCT GAG GAG GAA ATC TGG GAG 1296

GAG CTG GGT GTG ATG GGG GTG TAT GAT GGG AGG GAG CAC ACT 1338
GTC TAT GGG GAG CCC AGG AAA CTG CTC ACC CAA GAT TGG GTG 1380
CAG GAA AAC TAC CTG GAG TAC CGG CAG GTA CCC GGC AGT AAT 1422
CCT GCG CGC TAT GAG TTC CTG TGG GGT CCA AGG GCT CTG GCT 1464
GAA ACC AGC TAT GTG AAA GTC CTG GAG CAT GTG GTC AGG GTC 1506
AAT GCA AGA GTT CGC ATT GCC TAC CCA TCC CTG CGT GAA GCA 1548
GCT TTG TTA GAG GAG GAA GAG GGA GTC TGA 1578
GCATGAGTTG CAGCCAGGGC TGTGGGGAAG GGGCAGGGCT GGGCCAGTGC 1628
ATCTAACAGC CCTGTGCAGC AGCTTCCCTT GCCTCGTGTA ACATGAGGCC 1678
CATTCTTCAC TCTGTTTGAA GAAAATAGTC AGTGTTCTTA GTAGTGGGTT 1728
TCTATTTTGT TGGATGACTT GGAGATTTAT CTCTGTTTCC TTTTACAATT 1778
GTTGAAATGT TCCTTTTAAT GGATGGTTGA ATTAACTTCA GCATCCAAGT 1828
TTATGAATCG TAGTTAACGT ATATTGCTGT TAATATAGTT TAGGAGTAAG 1878
AGTCTTGTTT TTTATTCAGA TTGGGAAATC CGTTCTATTT TGTGAATTTG 1928
GGACATAATA ACAGCAGTGG AGTAAGTATT TAGAAGTGTG AATTCACCGT 1978
GAAATAGGTG AGATAAATTA AAAGATACTT AATTCCCGCC TTATGCCTCA 2028
GTCTATTCTG TAAAATTTAA AAATATATAT GCATACCTGG ATTTCCTTGG 2078
CTTCGTGAAT GTAAGAGAAA TTAAATCTGA ATAAATAATT CTTTCTGTTA 2128
ACTGGCTCAT TTCTTCTCTA TGCACTGAGC ATCTGCTCTG TGGAAGGCCC 2178
AGGATTAGTA GTGGAGATAC TAGGGTAAGC CAGACACACA CCTACCGATA 2228
GGGTATTAAG AGTCTAGGAG CGCGGTCATA TAATTAAGGT GACAAGATGT 2278
CCTCTAAGAT GTAGGGGAAA AGTAACGAGT GTGGGTATGG GGCTCCAGGT 2328
GAGAGTGGTC GGGTGTAAAT TCCCTGTGTG GGGCCTTTTG GGCTTTGGGA 2378
AACTGCATTT TCTTCTGAGG GATCTGATTC TAATGAAGCT TGGTGGGTCC 2428
AGGGCCAGAT TCTCAGAGGG AGAGGGAAAA GCCCAGATTG GAAAAGTTGC 2478
TCTGAGCAGT TCCTTTGTGA CAATGGATGA ACAGAGAGGA GCCTCTACCT 2528
GGG 2531

(2) INFORMATION FOR SEQUENCE ID NO: 14:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2531 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: genomic DNA
(ix) FEATURE:
(A) NAME/KEY: MAGE-41 gene
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

GGATCCAGGC CCTGCCTGGA GAAATGTGAG GGCCCTGAGT GAACACAGTG 50
GGGATCATCC ACTCCATGAG AGTGGGGACC TCACAGAGTC CAGCCTACCC 100
TCTTGATGGC ACTGAGGGAC CGGGGCTGTG CTTACAGTCT GCACCCTAAG 150
GGCCCATGGA TTCCTCTCCT AGGAGCTCCA GGAACAAGGC AGTGAGGCCT 200
TGGTCTGAGA CAGTGTCCTC AGGTTACAGA GCAGAGGATG CACAGGCTGT 250
GCCAGCAGTG AATGTTTGCC CTGAATGCAC ACCAAGGGCC CCACCTGCCA 300
CAAGACACAT AGGACTCCAA AGAGTCTGGC CTCACCTCCC TACCATCAAT 350
CCTGCAGAAT CGACCTCTGC TGGCCGGCTA TACCCTGAGG TGCTCTCTCA 400
CTTCCTCCTT CAGGTTCTGA GCAGACAGGC CAACCGGAGA CAGGATTCCC 450
TGGAGGCCAC AGAGGAGCAC CAAGGAGAAG ATCTGTAAGT AAGCCTTTGT 500
TAGAGCCTCT AAGATTTGGT TCTCAGCTGA GGTCTCTCAC ATGCTCCCTC 550

TCTCCGTAGG CCTGTGGGTC CCCATTGCCC AGCTTTTGCC TGCACTCTTG 600
CCTGCTGCCC TGAGCAGAGT CATC 624
ATG TCT TCT GAG CAG AAG AGT CAG CAC TGC AAG CCT GAG GAA 666
GGC GTT GAG GCC CAA GAA GAG GCC CTG GGC CTG GTG GGT GCG 708
CAG GCT CCT ACT ACT GAG GAG CAG GAG GCT GCT GTC TCC TCC 750
TCC TCT CCT CTG GTC CCT GGC ACC CTG GAG GAA GTG CCT GCT 792
GCT GAG TCA GCA GGT CCT CCC CAG AGT CCT CAG GGA GCC TCT 834
GCC TTA CCC ACT ACC ATC AGC TTC ACT TGC TGG AGG CAA CCC 876
AAT GAG GGT TCC AGC AGC CAA GAA GAG GAG GGG CCA AGC ACC 918
TCG CCT GAC GCA GAG TCC TTG TTC CGA GAA GCA CTC AGT AAC 960
AAG GTG GAT GAG TTG GCT CAT TTT CTG CTC CGC AAG TAT CGA 1002
GCC AAG GAG CTG GTC ACA AAG GCA GAA ATG CTG GAG AGA GTC 1044
ATC AAA AAT TAC AAG CGC TGC TTT CCT GTG ATC TTC GGC AAA 1086
GCC TCC GAG TCC CTG AAG ATG ATC TTT GGC ATT GAC GTG AAG 1128
GAA GTG GAC CCC ACC AGC AAC ACC TAC ACC CTT GTC ACC TGC 1170
CTG GGC CTT TCC TAT GAT GGC CTG CTG GGT AAT AAT CAG ATC 1212
TTT CCC AAG ACA GGC CTT CTG ATA ATC GTC CTG GGC ACA ATT 1254
GCA ATG GAG GGC GAC AGC GCC TCT GAG GAG GAA ATC TGG GAG 1296
GAG CTG GGT GTG ATG GGG GTG TAT GAT GGG AGG GAG CAC ACT 1338
GTC TAT GGG GAG CCC AGG AAA CTG CTC ACC CAA GAT TGG GTG 1380
CAG GAA AAC TAC CTG GAG TAC CGG CAG GTA CCC GGC AGT AAT 1422
CCT GCG CGC TAT GAG TTC CTG TGG GGT CCA AGG GCT CTG GCT 1464
GAA ACC AGC TAT GTG AAA GTC CTG GAG CAT GTG GTC AGG GTC 1506
AAT GCA AGA GTT CGC ATT GCC TAC CCA TCC CTG CGT GAA GCA 1548
GCT TTG TTA GAG GAG GAA GAG GGA GTC TGA 1578
GCATGAGTTG CAGCCAGGGC TGTGGGGAAG GGGCAGGGCT GGGCCAGTGC 1628
ATCTAACAGC CCTGTGCAGC AGCTTCCCTT GCCTCGTGTA ACATGAGGCC 1678
CATTCTTCAC TCTGTTTGAA GAAAATAGTC AGTGTTCTTA GTAGTGGGTT 1728
TCTATTTTGT TGGATGACTT GGAGATTTAT CTCTGTTTCC TTTTACAATT 1778
GTTGAAATGT TCCTTTTAAT GGATGGTTGA ATTAACTTCA GCATCCAAGT 1828
TTATGAATCG TAGTTAACGT ATATTGCTGT TAATATAGTT TAGGAGTAAG 1878
AGTCTTGTTT TTTATTCAGA TTGGGAAATC CGTTCTATTT TGTGAATTTG 1928
GGACATAATA ACAGCAGTGG AGTAAGTATT TAGAAGTGTG AATTCACCGT 1978
GAAATAGGTG AGATAAATTA AAAGATACTT AATTCCCGCC TTATGCCTCA 2028
GTCTATTCTG TAAAATTTAA AAATATATAT GCATACCTGG ATTTCCTTGG 2078
CTTCGTGAAT GTAAGAGAAA TTAAATCTGA ATAAATAATT CTTTCTGTTA 2128
ACTGGCTCAT TTCTTCTCTA TGCACTGAGC ATCTGCTCTG TGGAAGGCCC 2178
AGGATTAGTA GTGGAGATAC TAGGGTAAGC CAGACACACA CCTACCGATA 2228
GGGTATTAAG AGTCTAGGAG CGCGGTCATA TAATTAAGGT GACAAGATGT 2278
CCTCTAAGAT GTAGGGGAAA AGTAACGAGT GTGGGTATGG GGCTCCAGGT 2328
GAGAGTGGTC GGGTGTAAAT TCCCTGTGTG GGGCCTTTTG GGCTTTGGGA 2378
AACTCCATTT TCTTCTGAGG GATCTGATTC TAATGAAGCT TGGTGGGTCC 2428
AGGGCCAGAT TCTCAGAGGG AGAGGGAAAA GCCCAGATTG GAAAAGTTGC 2478
TCTGAGCGGT TCCTTTGTGA CAATGGATGA ACAGAGAGGA GCCTCTACCT 2528
GGG 2531

(2) INFORMATION FOR SEQUENCE ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1068 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(ix) FEATURE:

(A) NAME/KEY: cDNA MAGE-4

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

```
  G GGG CCA AGC ACC TCG CCT GAC GCA GAG TCC TTG TTC CGA          40
GAA GCA CTC AGT AAC AAG GTG GAT GAG TTG GCT CAT TTT CTG          82
CTC CGC AAG TAT CGA GCC AAG GAG CTG GTC ACA AAG GCA GAA         124
ATG CTG GAG AGA GTC ATC AAA AAT TAC AAG CGC TGC TTT CCT         166
GTG ATC TTC GGC AAA GCC TCC GAG TCC CTG AAG ATG ATC TTT         208
GGC ATT GAC GTG AAG GAA GTG GAC CCC GCC AGC AAC ACC TAC         250
ACC CTT GTC ACC TGC CTG GGC CTT TCC TAT GAT GGC CTG CTG         292
GGT AAT AAT CAG ATC TTT CCC AAG ACA GGC CTT CTG ATA ATC         334
GTC CTG GGC ACA ATT GCA ATG GAG GGC GAC AGC GCC TCT GAG         376
GAG GAA ATC TGG GAG GAG CTG GGT GTG ATG GGG GTG TAT GAT         418
GGG AGG GAG CAC ACT GTC TAT GGG GAG CCC AGG AAA CTG CTC         460
ACC CAA GAT TGG GTG CAG GAA AAC TAC CTG GAG TAC CGG CAG         502
GTA CCC GGC AGT AAT CCT GCG CGC TAT GAG TTC CTG TGG GGT         544
CCA AGG GCT CTG GCT GAA ACC AGC TAT GTG AAA GTC CTG GAG         586
CAT GTG GTC AGG GTC AAT GCA AGA GTT CGC ATT GCC TAC CCA         628
TCC CTG CGT GAA GCA GCT TTG TTA GAG GAG GAA GAG GGA GTC         670
TGAGCATGAG TTGCAGCCAG GGCTGTGGGG AAGGGGCAGG GCTGGGCCAG         720
TGCATCTAAC AGCCCTGTGC AGCAGCTTCC CTTGCCTCGT GTAACATGAG         770
GCCCATTCTT CACTCTGTTT GAAGAAAATA GTCAGTGTTC TTAGTAGTGG         820
GTTTCTATTT TGTTGGATGA CTTGGAGATT TATCTCTGTT TCCTTTTACA         870
ATTGTTGAAA TGTTCCTTTT AATGGATGGT TGAATTAACT TCAGCATCCA         920
AGTTTATGAA TCGTAGTTAA CGTATATTGC TGTTAATATA GTTTAGGAGT         970
AAGAGTCTTG TTTTTTATTC AGATTGGGAA ATCCGTTCTA TTTTGTGAAT        1020
TTGGGACATA ATAACAGCAG TGGAGTAAGT ATTTAGAAGT GTGAATTC          1068
```

(2) INFORMATION FOR SEQUENCE ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 2226 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

(ix) FEATURE:

(A) NAME/KEY: MAGE-5 gene

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

```
GGATCCAGGC CTTGCCAGGA GAAAGGTGAG GGCCCTGTGT GAGCACAGAG          50
GGGACCATTC ACCCCAAGAG GGTGGAGACC TCACAGATTC CAGCCTACCC         100
```

TCCTGTTAGC ACTGGGGGCC TGAGGCTGTG CTTGCAGTCT GCACCCTGAG 150
GGCCCATGCA TTCCTCTTCC AGGAGCTCCA GGAAACAGAC ACTGAGGCCT 200
TGGTCTGAGG CCGTGCCCTC AGGTCACAGA GCAGAGGAGA TGCAGACGTC 250
TAGTGCCAGC AGTGAACGTT TGCCTTGAAT GCACACTAAT GGCCCCCATC 300
GCCCCAGAAC ATATGGGACT CCAGAGCACC TGGCCTCACC CTCTCTACTG 350
TCAGTCCTGC AGAATCAGCC TCTGCTTGCT TGTGTACCCT GAGGTGCCCT 400
CTCACTTTTT CCTTCAGGTT CTCAGGGGAC AGGCTGACCA GGATCACCAG 450
GAAGCTCCAG AGGATCCCCA GGAGGCCCTA GAGGAGCACC AAAGGAGAAG 500
ATCTGTAAGT AAGCCTTTGT TAGAGCCTCC AAGGTTCAGT TTTTAGCTGA 550
GGCTTCTCAC ATGCTCCCTC TCTCTCCAGG CCAGTGGGTC TCCATTGCCC 600
AGCTCCTGCC CACACTCCTG CCTGTTGCGG TGACCAGAGT CGTC 644
ATG TCT CTT GAG CAG AAG AGT CAG CAC TGC AAG CCT GAG GAA 686
CTC CTC TGG TCC CAG GCA CCC TGG GGG AGG TGC CTG CTG CTG 728
GGT CAC CAG GTC CTC TCA AGA GTC CTC AGG GAG CCT CCG CCA 770
TCC CCA CTG CCA TCG ATT TCA CTC TAT GGA GGC AAT CCA TTA 812
AGG GCT CCA GCA ACC AAG AAG AGG AGG GGC CAA GCA CCT CCC 854
CTG ACC CAG AGT CTG TGT TCC GAG CAG CAC TCA GTA AGA AGG 896
TGG CTG ACT TGA 908
TTCATTTTCT GCTCCTCAAG TATTAAGTCA AGGAGCTGGT CACAAAGGCA 958
GAAATGCTGG AGAGCGTCAT CAAAAATTAC AAGCGCTGCT TTCCTGAGAT 1008
CTTCGGCAAA GCCTCCGAGT CCTTGCAGCT GGTCTTTGGC ATTGACGTGA 1058
AGGAAGCGGA CCCCACCAGC AACACCTACA CCCTTGTCAC CTGCCTGGGA 1108
CTCCTATGAT GGCCTGCTGG TTGATAATAA TCAGATCATG CCCAAGACGG 1158
GCCTCCTGAT AATCGTCTTG GGCATGATTG CAATGGAGGG CAAATGCGTC 1208
CCTGAGGAGA AAATCTGGGA GGAGCTGAGT GTGATGAAGG TGTATGTTGG 1258
GAGGGAGCAC AGTGTCTGTG GGGAGCCCAG GAAGCTGCTC ACCCAAGATT 1308
TGGTGCAGGA AAACTACCTG GAGTACCGGC AGGTGCCCAG CAGTGATCCC 1358
ATATGCTATG AGTTACTGTG GGGTCCAAGG GCACTCGCTG CTTGAAAGTA 1408
CTGGAGCACG TGGTCAGGGT CAATGCAAGA GTTCTCATTT CCTACCCATC 1458
CCTGCGTGAA GCAGCTTTGA GAGAGGAGGA AGAGGGAGTC TGAGCATGAG 1508
CTGCAGCCAG GGCCACTGCG AGGGGGGCTG GGCCAGTGCA CCTTCCAGGG 1558
CTCCGTCCAG TAGTTTCCCC TGCCTTAATG TGACATGAGG CCCATTCTTC 1608
TCTCTTTGAA GAGAGCAGTC AACATTCTTA GTAGTGGGTT TCTGTTCTAT 1658
TGGATGACTT TGAGATTTGT CTTTGTTTCC TTTTGGAATT GTTCAAATGT 1708
TTCTTTTAAT GGGTGGTTGA ATGAACTTCA GCATTCAAAT TTATGAATGA 1758
CAGTAGTCAC ACATAGTGCT GTTTATATAG TTTAGGAGTA AGAGTCTTGT 1808
TTTTTATTCA GATTGGGAAA TCCATTCCAT TTTGTGAATT GGGACATAGT 1858
TACAGCAGTG GAATAAGTAT TCATTTAGAA ATGTGAATGA GCAGTAAAAC 1908
TGATGACATA AAGAAATTAA AAGATATTTA ATTCTTGCTT ATACTCAGTC 1958
TATTCGGTAA AATTTTTTTT AAAAAATGTG CATACCTGGA TTTCCTTGGC 2008
TTCTTTGAGA ATGTAAGACA AATTAAATCT GAATAAATCA TTCTCCCTGT 2058
TCACTGGCTC ATTTATTCTC TATGCACTGA GCATTTGCTC TGTGGAAGGC 2108
CCTGGGTTAA TAGTGGAGAT GCTAAGGTAA GCCAGACTCA CCCCTACCCA 2158
CAGGGTAGTA AAGTCTAGGA GCAGCAGTCA TATAATTAAG GTGGAGAGAT 2208
GCCCTCTAAG ATGTAGAG 2226

(2) INFORMATION FOR SEQUENCE ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 2305 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

(ix) FEATURE:

(A) NAME/KEY: MAGE-51 gene

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

```
GGATCCAGGC CTTGCCAGGA GAAAGGTGAG GGCCCTGTGT GAGCACAGAG        50
GGGACCATTC ACCCCAAGAG GGTGGAGACC TCACAGATTC CAGCCTACCC       100
TCCTGTTAGC ACTGGGGGCC TGAGGCTGTG CTTGCAGTCT GCACCCTGAG       150
GGCCCATGCA TTCCTCTTCC AGGAGCTCCA GGAAACAGAC ACTGAGGCCT       200
TGGTCTGAGG CCGTGCCCTC AGGTCACAGA GCAGAGGAGA TGCAGACGTC       250
TAGTGCCAGC AGTGAACGTT TGCCTTGAAT GCACACTAAT GGCCCCCATC       300
GCCCCAGAAC ATATGGGACT CCAGAGCACC TGGCCTCACC CTCTCTACTG       350
TCAGTCCTGC AGAATCAGCC TCTGCTTGCT TGTGTACCCT GAGGTGCCCT       400
CTCACTTTTT CCTTCAGGTT CTCAGGGGAC AGGCTGACCA GGATCACCAG       450
GAAGCTCCAG AGGATCCCCA GGAGGCCCTA GAGGAGCACC AAAGGAGAAG       500
ATCTGTAAGT AAGCCTTTGT TAGAGCCTCC AAGGTTCAGT TTTTAGCTGA       550
GGCTTCTCAC ATGCTCCCTC TCTCTCCAGG CCAGTGGGTC TCCATTGCCC       600
AGCTCCTGCC CACACTCCTG CCTGTTGCGG TGACCAGAGT CGTC             644
ATG TCT CTT GAG CAG AAG AGT CAG CAC TGC AAG CCT GAG GAA      686
GGC CTT GAC ACC CAA GAA GAG CCC TGG GCC TGG TGG GTG TGC      728
AGG CTG CCA CTA CTG AGG AGC AGG AGG CTG TGT CCT CCT CCT      770
CTC CTC TGG TCC CAG GCA CCC TGG GGG AGG TGC CTG CTG CTG      812
GGT CAC CAG GTC CTC TCA AGA GTC CTC AGG GAG CCT CCG CCA      854
TCC CCA CTG CCA TCG ATT TCA CTC TAT GGA GGC AAT CCA TTA      896
AGG GCT CCA GCA ACC AAG AAG AGG AGG GGC CAA GCA CCT CCC      938
CTG ACC CAG AGT CTG TGT TCC GAG CAG CAC TCA GTA AGA AGG      980
TGG CTG ACT TGA                                              992
TTCATTTTCT GCTCCTCAAG TATTAAGTCA AGGAGCCGGT CACAAAGGCA      1042
GAAATGCTGG AGAGCGTCAT CAAAAATTAC AAGCGCTGCT TTCCTGAGAT      1092
CTTCGGCAAA GCCTCCGAGT CCTTGCAGCT GGTCTTTGGC ATTGACGTGA      1142
AGGAAGCGGA CCCCACCAGC AACACCTACA CCCTTGTCAC CTGCCTGGGA      1192
CTCCTATGAT GGCCTGGTGG TTTAATCAGA TCATGCCCAA GACGGGCCTC      1242
CTGATAATCG TCTTGGGCAT GATTGCAATG GAGGGCAAAT GCGTCCCTGA      1292
GGAGAAAATC TGGGAGGAGC TGGGTGTGAT GAAGGTGTAT GTTGGGAGGG      1342
AGCACAGTGT CTGTGGGGAG CCCAGGAAGC TGCTCACCCA AGATTTGGTG      1392
CAGGAAAACT ACCTGGAGTA CCGCAGGTGC CCAGCAGTGA TCCCATATGC      1442
TATGAGTTAC TGTGGGGTCC AAGGGCACTC GCTGCTTGAA AGTACTGGAG      1492
CACGTGGTCA GGGTCAATGC AAGAGTTCTC ATTTCCTACC CATCCCTGCA      1542
TGAAGCAGCT TTGAGAGAGG AGGAAGAGGG AGTCTGAGCA TGAGCTGCAG      1592
CCAGGGCCAC TGCGAGGGGG GCTGGGCCAG TGCACCTTCC AGGGCTCCGT      1642
CCAGTAGTTT CCCCTGCCTT AATGTGACAT GAGGCCCATT CTTCTCTCTT      1692
TGAAGAGAGC AGTCAACATT CTTAGTAGTG GGTTTCTGTT CTATTGGATG      1742
ACTTTGAGAT TTGTCTTTGT TTCCTTTTGG AATTGTTCAA ATGTTCCTTT      1792
TAATGGGTGG TTGAATGAAC TTCAGCATTC AAATTTATGA ATGACAGTAG      1842
TCACACATAG TGCTGTTTAT ATAGTTTAGG AGTAAGAGTC TTGTTTTTTA      1892
TTCAGATTGG GAAATCCATT CCATTTTGTG AATTGGGACA TAGTTACAGC      1942
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AGTGGAATAA GTATTCATTT AGAAATGTGA ATGAGCAGTA AAACTGATGA 1992
GATAAAGAAA TTAAAAGATA TTTAATTCTT GCCTTATACT CAGTCTATTC 2042
GGTAAAATTT TTTTTTAAAA ATGTGCATAC CTGGATTTCC TTGGCTTCTT 2092
TGAGAATGTA AGACAAATTA AATCTGAATA AATCATTCTC CCTGTTCACT 2142
GGCTCATTTA TTCTCTATGC ACTGAGCATT TGCTCTGTGG AAGGCCCTGG 2192
GTTAATAGTG GAGATGCTAA GGTAAGCCAG ACTCACCCCT ACCCACAGGG 2242
TAGTAAAGTC TAGGAGCAGC AGTCATATAA TTAAGGTGGA GAGATGCCCT 2292
CTAAGATGTA GAG 2305

(2) INFORMATION FOR SEQUENCE ID NO: 18:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 225 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: cDNA
(ix) FEATURE:
(A) NAME/KEY: MAGE-6 gene
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

TAT TTC TTT CCT GTG ATC TTC AGC AAA GCT TCC GAT TCC TTG 42
CAG CTG GTC TTT GGC ATC GAG CTG ATG GAA GTG GAC CCC ATC 84
GGC CAC GTG TAC ATC TTT GCC ACC TGC CTG GGC CTC TCC TAC 126
GAT GGC CTG CTG GGT GAC AAT CAG ATC ATG CCC AGG ACA GGC 168
TTC CTG ATA ATC ATC CTG GCC ATA ATC GCA AGA GAG GGC GAC 210
TGT GCC CCT GAG GAG 225

(2) INFORMATION FOR SEQUENCE ID NO: 19:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1947 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: genomic DNA
(ix) FEATURE:
(A) NAME/KEY: MAGE-7 gene
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

TGAATGGACA ACAAGGGCCC CACACTCCCC AGAACACAAG GGACTCCAGA 50
GAGCCCAGCC TCACCTTCCC TACTGTCAGT CCTGCAGCCT CAGCCTCTGC 100
TGGCCGGCTG TACCCTGAGG TGCCCTCTCA CTTCCTCCTT CAGGTTCTCA 150
GCGGACAGGC CGGCCAGGAG GTCAGAAGCC CCAGGAGGCC CCAGAGGAGC 200
ACCGAAGGAG AAGATCTGTA AGTAGGCCTT TGTTAGGGCC TCCAGGGCGT 250
GGTTCACAAA TGAGGCCCCT CACAAGCTCC TTCTCTCCCC AGATCTGTGG 300
GTTCCTCCCC ATCGCCCAGC TGCTGCCCGC ACTCCAGCCT GCTGCCCTGA 350
CCAGAGTCAT CATGTCTTCT GAGCAGAGGA GTCAGCACTG CAAGCCTGAG 400

GATGCCTTGA GGCCCAAGGA CAGGAGGCTC TGGGCCTGGT GGGTGCGCAG 450
GCTCCCGCCA CCGAGGAGCA CGAGGCTGCC TCCTCCTTCA CTCTGATTGA 500
AGGCACCCTG GAGGAGGTGC CTGCTGCTGG GTCCCCCAGT CCTCCCCTGA 550
GTCTCAGGGT TCCTCCTTTT CCCTGACCAT CAGCAACAAC ACTCTATGGA 600
GCCAATCCAG TGAGGGCACC AGCAGCCGGG AAGAGGAGGG GCCAACCACC 650
TAGACACACC CCGCTCACCT GGCGTCCTTG TTCCA 685
ATG GGA AGG TGG CTG AGT TGG TTC GCT TCC TGC TGC ACA AGT 727
ATC GAG TCA AGG AGC TGG TCA CAA AGG CAG AAA TGC TGG ACA 769
GTG TCA TCA AAA ATT ACA AGC ACT AGT TTC CTT GTG ATC TAT 811
GGC AAA GCC TCA GAG TGC ATG CAG GTG ATG TTT GGC ATT GAC 853
ATG AAG GAA GTG GAC CCC GCG GCC ACT CCT ACG TCC TTG TCA 895
CCT GCT TGG GCC TCT CCT ACA ATG GCC TGC TGG GTG ATG ATC 937
AGA GCA TGC CCG AGA CCG GCC TTC TGA 964
TTATGGTCTT GACCATGATC TTAATGGAGG GCCACTGTGC CCCTGAGGAG 1014
GCAATCTGGG AAGCGTTGAG TGTAATGGTG TATGATGGGA TGGAGCAGTT 1064
TCTTTGGGCA GCTGAGGAAG CTGCTCACCC AAGATTGGGT GCAGGAAAAC 1114
TACCTGCAAT ACCGCCAGGT GCCCAGCAGT GATCCCCCGT GCTACCAGTT 1164
CCTGTGGGGT CCAAGGGCCC TCATTGAAAC CAGCTATGTG AAAGTCCTGG 1214
AGTATGCAGC CAGGGTCAGT ACTAAAGAGA GCATTTCCTA CCCATCCCTG 1264
CATGAAGAGG CTTTGGGAGA GGAGGAAGAG GGAGTCTGAG CAGAAGTTGC 1314
AGCCAGGGCC AGTGGGGCAG ATTGGGGGAG GGCCTGGGCA GTGCACGTTC 1364
CACACATCCA CCACCTTCCC TGTCCTGTTA CATGAGGCCC ATTCTTCACT 1414
CTGTGTTTGA AGAGAGCAGT CAATGTTCTC AGTAGCGGGG AGTGTGTTGG 1464
GTGTGAGGGA ATACAAGGTG GACCATCTCT CAGTTCCTGT TCTCTTGGGC 1514
GATTTGGAGG TTTATCTTTG TTTCCTTTTG CAGTCGTTCA AATGTTCCTT 1564
TTAATGGATG GTGTAATGAA CTTCAACATT CATTTCATGT ATGACAGTAG 1614
GCAGACTTAC TGTTTTTTAT ATAGTTAAAA GTAAGTGCAT TGTTTTTTAT 1664
TTATGTAAGA AAATCTATGT TATTTCTTGA ATTGGGACAA CATAACATAG 1714
CAGAGGATTA AGTACCTTTT ATAATGTGAA AGAACAAAGC GGTAAAATGG 1764
GTGAGATAAA GAAATAAAGA AATTAAATTG GCTGGGCACG GTGGCTCACG 1814
CCTGTAATCC CAGCACTTTA GGAGGCAGAG GCACGGGGAT CACGAGGTCA 1864
GGAGATCGAG ACCATTCTGG CTAACACAGT GAAACACCAT CTCTATTAAA 1914
AATACAAAAC TTAGCCGGGC GTGGTGGCGG GTG 1947

(2) INFORMATION FOR SEQUENCE ID NO: 20:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1810 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: genomic DNA
(ix) FEATURE:
(A) NAME/KEY: MAGE-8 gene
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

GAGCTCCAGG AACCAGGCTG TGAGGTCTTG GTCTGAGGCA GTATCTTCAA 50
TCACAGAGCA TAAGAGGCCC AGGCAGTAGT AGCAGTCAAG CTGAGGTGGT 100
GTTTCCCCTG TATGTATACC AGAGGCCCCT CTGGCATCAG AACAGCAGGA 150
ACCCCACAGT TCCTGGCCCT ACCAGCCCTT TTGTCAGTCC TGGAGCCTTG 200
GCCTTTGCCA GGAGGCTGCA CCCTGAGATG CCCTCTCAAT TTCTCCTTCA 250
GGTTCGCAGA GAACAGGCCA GCCAGGAGGT CAGGAGGCCC CAGAGAAGCA 300

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CTGAAGAAGA CCTGTAAGTA GACCTTTGTT AGGGCATCCA GGGTGTAGTA          350
CCCAGCTGAG GCCTCTCACA CGCTTCCTCT CTCCCCAGGC CTGTGGGTCT          400
CAATTGCCCA GCTCCGGCCC ACACTCTCCT GCTGCCCTGA CCTGAGTCAT          450
C                                                               451
ATG CTT CTT GGG CAG AAG AGT CAG CGC TAC AAG GCT GAG GAA          493
GGC CTT CAG GCC CAA GGA GAG GCA CCA GGG CTT ATG GAT GTG          535
CAG ATT CCC ACA GCT GAG GAG CAG AAG GCT GCA TCC TCC TCC          577
TCT ACT CTG ATC ATG GGA ACC CTT GAG GAG GTG ACT GAT TCT          619
GGG TCA CCA AGT CCT CCC CAG AGT CCT GAG GGT GCC TCC TCT          661
TCC CTG ACT GTC ACC GAC AGC ACT CTG TGG AGC CAA TCC GAT          703
GAG GGT TCC AGC AGC AAT GAA GAG GAG GGG CCA AGC ACC TCC          745
CCG GAC CCA GCT CAC CTG GAG TCC CTG TTC CGG GAA GCA CTT          787
GAT GAG AAA GTG GCT GAG TTA GTT CGT TTC CTG CTC CGC AAA          829
TAT CAA ATT AAG GAG CCG GTC ACA AAG GCA GAA ATG CTT GAG          871
AGT GTC ATC AAA AAT TAC AAG AAC CAC TTT CCT GAT ATC TTC          913
AGC AAA GCC TCT GAG TGC ATG CAG GTG ATC TTT GGC ATT GAT          955
GTG AAG GAA GTG GAC CCT GCC GGC CAC TCC TAC ATC CTT GTC          997
ACC TGC CTG GGC CTC TCC TAT GAT GGC CTG CTG GGT GAT GAT         1039
CAG AGT ACG CCC AAG ACC GGC CTC CTG ATA ATC GTC CTG GGC         1081
ATG ATC TTA ATG GAG GGC AGC CGC GCC CCG GAG GAG GCA ATC         1123
TGG GAA GCA TTG AGT GTG ATG GGG GCT GTA TGA                     1156
TGGGAGGGAG CACAGTGTCT ATTGGAAGCT CAGGAAGCTG CTCACCCAAG          1206
AGTGGGTGCA GGAGAACTAC CTGGAGTACC GCCAGGCGCC CGGCAGTGAT          1256
CCTGTGCGCT ACGAGTTCCT GTGGGGTCCA AGGGCCCTTG CTGAAACCAG         1306
CTATGTGAAA GTCCTGGAGC ATGTGGTCAG GGTCAATGCA AGAGTTCGCA         1356
TTTCCTACCC ATCCCTGCAT GAAGAGGCTT TGGGAGAGGA GAAAGGAGTT         1406
TGAGCAGGAG TTGCAGCTAG GGCCAGTGGG GCAGGTTGTG GGAGGGCCTG         1456
GGCCAGTGCA CGTTCCAGGG CCACATCCAC CACTTTCCCT GCTCTGTTAC         1506
ATGAGGCCCA TTCTTCACTC TGTGTTTGAA GAGAGCAGTC ACAGTTCTCA         1556
GTAGTGGGGA GCATGTTGGG TGTGAGGGAA CACAGTGTGG ACCATCTCTC         1606
AGTTCCTGTT CTATTGGGCG ATTTGGAGGT TTATCTTTGT TTCCTTTTGG         1656
AATTGTTCCA ATGTTCCTTC TAATGGATGG TGTAATGAAC TTCAACATTC         1706
ATTTTATGTA TGACAGTAGA CAGACTTACT GCTTTTTATA TAGTTTAGGA         1756
GTAAGAGTCT TGCTTTTCAT TTATACTGGG AAACCCATGT TATTTCTTGA         1806
ATTC                                                          1810
```

(2) INFORMATION FOR SEQUENCE ID NO: 21:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1412 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

(ix) FEATURE:

(A) NAME/KEY: MAGE-9 gene

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

```
TCTGAGACAG TGTCCTCAGG TCGCAGAGCA GAGGAGACCC AGGCAGTGTC           50
AGCAGTGAAG GTGAAGTGTT CACCCTGAAT GTGCACCAAG GGCCCCACCT          100
GCCCCAGCAC ACATGGGACC CCATAGCACC TGGCCCCATT CCCCCTACTG          150
TCACTCATAG AGCCTTGATC TCTGCAGGCT AGCTGCACGC TGAGTAGCCC          200
```

TCTCACTTCC TCCCTCAGGT TCTCGGGACA GGCTAACCAG GAGGACAGGA 250
GCCCCAAGAG GCCCCAGAGC AGCACTGACG AAGACCTGTA AGTCAGCCTT 300
TGTTAGAACC TCCAAGGTTC GGTTCTCAGC TGAAGTCTCT CACACACTCC 350
CTCTCTCCCC AGGCCTGTGG GTCTCCATCG CCCAGCTCCT GCCCACGCTC 400
CTGACTGCTG CCCTGACCAG AGTCATC 427
ATG TCT CTC GAG CAG AGG AGT CCG CAC TGC AAG CCT GAT GAA 469
GAC CTT GAA GCC CAA GGA GAG GAC TTG GGC CTG ATG GGT GCA 511
CAG GAA CCC ACA GGC GAG GAG GAG GAG ACT ACC TCC TCC TCT 553
GAC AGC AAG GAG GAG GAG GTG TCT GCT GCT GGG TCA TCA AGT 595
CCT CCC CAG AGT CCT CAG GGA GGC GCT TCC TCC TCC ATT TCC 637
GTC TAC TAC ACT TTA TGG AGC CAA TTC GAT GAG GGC TCC AGC 679
AGT CAA GAA GAG GAA GAG CCA AGC TCC TCG GTC GAC CCA GCT 721
CAG CTG GAG TTC ATG TTC CAA GAA GCA CTG AAA TTG AAG GTG 763
GCT GAG TTG GTT CAT TTC CTG CTC CAC AAA TAT CGA GTC AAG 805
GAG CCG GTC ACA AAG GCA GAA ATG CTG GAG AGC GTC ATC AAA 847
AAT TAC AAG CGC TAC TTT CCT GTG ATC TTC GGC AAA GCC TCC 889
GAG TTC ATG CAG GTG ATC TTT GGC ACT GAT GTG AAG GAG GTG 931
GAC CCC GCC GGC CAC TCC TAC ATC CTT GTC ACT GCT CTT GGC 973
CTC TCG TGC GAT AGC ATG CTG GGT GAT GGT CAT AGC ATG CCC 1015
AAG GCC GCC CTC CTG ATC ATT GTC CTG GGT GTG ATC CTA ACC 1057
AAA GAC AAC TGC GCC CCT GAA GAG GTT ATC TGG GAA GCG TTG 1099
AGT GTG ATG GGG GTG TAT GTT GGG AAG GAG CAC ATG TTC TAC 1141
GGG GAG CCC AGG AAG CTG CTC ACC CAA GAT TGG GTG CAG GAA 1183
AAC TAC CTG GAG TAC CGG CAG GTG CCC GGC AGT GAT CCT GCG 1225
CAC TAC GAG TTC CTG TGG GGT TCC AAG GCC CAC GCT GAA ACC 1267
AGC TAT GAG AAG GTC ATA AAT TAT TTG GTC ATG CTC AAT GCA 1309
AGA GAG CCC ATC TGC TAC CCA TCC CTT TAT GAA GAG GTT TTG 1351
GGA GAG GAG CAA GAG GGA GTC TGA 1375
GCACCAGCCG CAGCCGGGGC CAAAGTTTGT GGGGTCA 1412

(2) INFORMATION FOR SEQUENCE ID NO: 22:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 920 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: genomic DNA
(ix) FEATURE:
(A) NAME/KEY: MAGE-10 gene
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

ACCTGCTCCA GGACAAAGTG GACCCCACTG CATCAGCTCC ACCTACCCTA 50
CTGTCAGTCC TGGAGCCTTG GCCTCTGCCG GCTGCATCCT GAGGAGCCAT 100
CTCTCACTTC CTTCTTCAGG TTCTCAGGGG ACAGGGAGAG CAAGAGGTCA 150
AGAGCTGTGG GACACCACAG AGCAGCACTG AAGGAGAAGA CCTGTAAGTT 200
GGCCTTTGTT AGAACCTCCA GGGTGTGGTT CTCAGCTGTG GCCACTTACA 250
CCCTCCCTCT CTCCCCAGGC CTGTGGGTCC CCATCGCCCA AGTCCTGCCC 300
ACACTCCCAC CTGCTACCCT GATCAGAGTC ATC 333
ATG CCT CGA GCT CCA AAG CGT CAG CGC TGC ATG CCT GAA GAA 375
GAT CTT CAA TCC CAA AGT GAG ACA CAG GGC CTC GAG GGT GCA 417
CAG GCT CCC CTG GCT GTG GAG GAG GAT GCT TCA TCA TCC ACT 459

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TCC ACC AGC TCC TCT TTT CCA TCC TCT TTT CCC TCC TCC TCC          501
TCT TCC TCC TCC TCC TCC TGC TAT CCT CTA ATA CCA AGC ACC          543
CCA GAG GAG GTT TCT GCT GAT GAT GAG ACA CCA AAT CCT CCC          585
CAG AGT GCT CAG ATA GCC TGC TCC TCC CCC TCG GTC GTT GCT          627
TCC CTT CCA TTA GAT CAA TCT GAT GAG GGC TCC AGC AGC CAA          669
AAG GAG GAG AGT CCA AGC ACC CTA CAG GTC CTG CCA GAC AGT          711
GAG TCT TTA CCC AGA AGT GAG ATA GAT GAA AAG GTG ACT GAT          753
TTG GTG CAG TTT CTG CTC TTC AAG TAT CAA ATG AAG GAG CCG          795
ATC ACA AAG GCA GAA ATA CTG GAG AGT GTC ATA AAA AAT TAT          837
GAA GAC CAC TTC CCT TTG TTG TTT AGT GAA GCC TCC GAG TGC          879
ATG CTG CTG GTC TTT GGC ATT GAT GTA AAG GAA GTG GAT CC           920
```

(2) INFORMATION FOR SEQUENCE ID NO: 23:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1107 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

(ix) FEATURE:

(A) NAME/KEY: MAGE-11 gene

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

```
AGAGAACAGG CCAACCTGGA GGACAGGAGT CCCAGGAGAA CCCAGAGGAT           50
CACTGGAGGA GAACAAGTGT AAGTAGGCCT TTGTTAGATT CTCCATGGTT          100
CATATCTCAT CTGAGTCTGT TCTCACGCTC CCTCTCTCCC CAGGCTGTGG          150
GGCCCCATCA CCCAGATATT TCCCACAGTT CGGCCTGCTG ACCTAACCAG          200
AGTCATCATG CCTCTTGAGC AAAGAAGTCA GCACTGCAAG CCTGAGGAAG          250
CCTTCAGGCC CAAGAAGAAG ACCTGGGCCT GGTGGGTGCA CAGGCTCTCC          300
AAGCTGAGGA GCAGGAGGCT GCCTTCTTCT CCTCTACTCT GAATGTGGGC          350
ACTCTAGAGG AGTTGCCTGC TGCTGAGTCA CCAAGTCCTC CCCAGAGTCC          400
TCAGGAAGAG TCCTTCTCTC CCACTGCCAT GGATGCCATC TTTGGGAGCC          450
TATCTGATGA GGGCTCTGGC AGCCAAGAAA AGGAGGGGCC AAGTACCTCG          500
CCTGACCTGA TAGACCCTGA GTCCTTTTCC CAAGATATAC TACATGACAA          550
GATAATTGAT TTGGTTCATT TATTCTCCGC AAGTATCGAG TCAAGGGGCT          600
GATCACAAAG GCAGAA                                              616
ATG CTG GGG AGT GTC ATC AAA AAT TAT GAG GAC TAC TTT CCT         658
GAG ATA TTT AGG GAA GCC TCT GTA TGC ATG CAA CTG CTC TTT         700
GGC ATT GAT GTG AAG GAA GTG GAC CCC ACT AGC CAC TCC TAT         742
GTC CTT GTC ACC TCC CTC AAC CTC TCT TAT GAT GGC ATA CAG         784
TGT AAT GAG CAG AGC ATG CCC AAG TCT GGC CTC CTG ATA ATA         826
GTC CTG GGT GTA ATC TTC ATG GAG GGG AAC TGC ATC CCT GAA         868
GAG GTT ATG TGG GAA GTC CTG AGC ATT ATG GGG GTG TAT GCT         910
GGA AGG GAG CAC TTC CTC TTT GGG GAG CCC AAG AGG CTC CTT         952
ACC CAA AAT TGG GTG CAG GAA AAG TAC CTG GTG TAC CGG CAG         994
GTG CCC GGC ACT GAT CCT GCA TGC TAT GAG TTC CTG TGG GGT        1036
CCA AGG GCC CAC GCT GAG ACC AGC AAG ATG AAA GTT CTT GAG        1078
TAC ATA GCC AAT GCC AAT GGG AGG GAT CC                         1107
```

(2) INFORMATION FOR SEQUENCE ID NO: 24:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 2150 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

(ix) FEATURE:

(A) NAME/KEY: smage-I

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

```
TCTGTCTGCA TATGCCTCCA CTTGTGTGTA GCAGTCTCAA ATGGATCTCT      50
CTCTACAGAC CTCTGTCTGT GTCTGGCACC CTAAGTGGCT TTGCATGGGC     100
ACAGGTTTCT GCCCCTGCAT GGAGCTTAAA TAGATCTTTC TCCACAGGCC     150
TATACCCCTG CATTGTAAGT TTAAGTGGCT TTATGTGGAT ACAGGTCTCT     200
GCCCTTGTAT GCAGGCCTAA GTTTTTCTGT CTGCTTAACC CCTCCAAGTG     250
AAGCTAGTGA AAGATCTAAC CCACTTTTGG AAGTCTGAAA CTAGACTTTT     300
ATGCAGTGGC CTAACAAGTT TTAATTTCTT CCACAGGGTT TGCAGAAAAG     350
AGCTTGATCC ACGAGTTCAG AAGTCCTGGT ATGTTCCTAG AAAG           394
ATG TTC TCC TGG AAA GCT TCA AAA GCC AGG TCT CCA TTA AGT    436
CCA AGG TAT TCT CTA CCT GGT AGT ACA GAG GTA CTT ACA GGT    478
TGT CAT TCT TAT CCT TCC AGA TTC CTG TCT GCC AGC TCT TTT    520
ACT TCA GCC CTG AGC ACA GTC AAC ATG CCT AGG GGT CAA AAG    562
AGT AAG ACC CGC TCC CGT GCA AAA CGA CAG CAG TCA CGC AGG    604
GAG GTT CCA GTA GTT CAG CCC ACT GCA GAG GAA GCA GGG TCT    646
TCT CCT GTT GAC CAG AGT GCT GGG TCC AGC TTC CCT GGT GGT    688
TCT GCT CCT CAG GGT GTG AAA ACC CCT GGA TCT TTT GGT GCA    730
GGT GTA TCC TGC ACA GGC TCT GGT ATA GGT GGT AGA AAT GCT    772
GCT GTC CTG CCT GAT ACA AAA AGT TCA GAT GGC ACC CAG GCA    814
GGG ACT TCC ATT CAG CAC ACA CTG AAA GAT CCT ATC ATG AGG    856
AAG GCT AGT GTG CTG ATA GAA TTC CTG CTA GAT AAA TTT AAG    898
ATG AAA GAA GCA GTT ACA AGG AGT GAA ATG CTG GCA GTA GTT    940
AAC AAG AAG TAT AAG GAG CAA TTC CCT GAG ATC CTC AGG AGA    982
ACT TCT GCA CGC CTA GAA TTA GTC TTT GGT CTT GAG TTG AAG   1024
GAA ATT GAT CCC AGC ACT CAT TCC TAT TTG CTG GTA GGC AAA   1066
CTG GGT CTT TCC ACT GAG GGA AGT TTG AGT AGT AAC TGG GGG   1108
TTG CCT AGG ACA GGT CTC CTA ATG TCT GTC CTA GGT GTG ATC   1150
TTC ATG AAG GGT AAC CGT GCC ACT GAG CAA GAG GTC TGG CAA   1192
TTT CTG CAT GGA GTG GGG GTA TAT GCT GGG AAG AAG CAC TTG   1234
ATC TTT GGC GAG CCT GAG GAG TTT ATA AGA GAT GTA GTG CGG   1276
GAA AAT TAC CTG GAG TAC CGC CAG GTA CCT GGC AGT GAT CCC   1318
CCA AGC TAT GAG TTC CTG TGG GGA CCC AGA GCC CAT GCT GAA   1360
ACA ACC AAG ATG AAA GTC CTG GAA GTT TTA GCT AAA GTC AAT   1402
GGC ACA GTC CCT AGT GCC TTC CCT AAT CTC TAC CAG TTG GCT   1444
CTT AGA GAT CAG GCA GGA GGG GTG CCA AGA AGG AGA GTT CAA   1486
GGC AAG GGT GTT CAT TCC AAG GCC CCA TCC CAA AAG TCC TCT   1528
AAC ATG TAG                                               1537
TTGAGTCTGT TCTGTTGTGT TTGAAAAACA GTCAGGCTCC TAATCAGTAG    1587
AGAGTTCATA GCCTACCAGA ACCAACATGC ATCCATTCTT GGCCTGTTAT    1637
ACATTAGTAG AATGGAGGCT ATTTTTGTTA CTTTTCAAAT GTTTGTTTAA    1687
CTAAACAGTG CTTTTTGCCA TGCTTCTTGT TAACTGCATA AAGAGGTAAC    1737
```

TGTCACTTGT CAGATTAGGA CTTGTTTTGT TATTTGCAAC AAACTGGAAA 1787
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GGAAAGTTTA TATTGTTAAT TTTGAAAATT TTATGAGTGT GATTGCTGTA 1937
TACTTTTTTC TTTTTTGTAT AATGCTAAGT GAAATAAAGT TGGATTTGAT 1987
GACTTTACTC AAATTCATTA GAAAGTAAAT CGTAAAACTC TATTACTTTA 2037
TTATTTTCTT CAATTATGAA TTAAGCATTG GTTATCTGGA AGTTTCTCCA 2087
GTAGCACAGG ATCTAGTATG AAATGTATCT AGTATAGGCA CTGACAGTGA 2137
GTTATCAGAG TCT 2150

(2) INFORMATION FOR SEQUENCE ID NO: 25:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2099 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: genomic DNA
(ix) FEATURE:
(A) NAME/KEY: smage-II
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

ACCTTATTGG GTCTGTCTGC ATATGCCTCC ACTTGTGTGT AGCAGTCTCA 50
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TAACAAGAAG TATAAGGAGC AATTCCCTGA GATCCTCAGG AGAACTTCTG 1000
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TGGCTCTTAG AGATCAGGCA GGAGGGGTGC CAAGAAGGAG AGTTCAAGGC 1500

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AAAGTTTATA TTGTTAGTTT TGAAAATTTT ATGAGTGTGA TTGCTGTATA      1950
CTTTTTTCTT TTTTGTATAA TGCTAAGTGA AATAAAGTTG GATTTGATGA      2000
CTTTACTCAA ATTCATTAGA AAGTAAATCA TAAAACTCTA TTACTTTATT      2050
ATTTTCTTCA ATTATTAATT AAGCATTGGT TATCTGGAAG TTTCTCCAG       2099
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(2) INFORMATION FOR SEQUENCE ID NO: 26:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 9 amino acids

(B) TYPE: amino acids

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:

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Glu Ala Asp Pro Thr Gly His Ser Tyr
 5
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TUMOR REJECTION ANTIGEN PRECURSORS, TUMOR REJECTION ANTIGENS AND USES THEREOF

This application is a continuation-in-part of Serial Number 807,043, filed December 12, 1991, which is a continuation-in-part of Serial Number 764,364, filed September 23, 1991, which is a continuation-in-part of Serial Number 728,838, filed July 9, 1991, which is a continuation-in-part of Serial Number 705,702, filed May 23, 1991, and now abandoned.

FIELD OF THE INVENTION

This invention relates in general to the field of immunogenetics as applied to the study of oncology. More specifically, it relates to the study and analysis of mechanisms by which tumors are recognized by the organism's immune system such as through the presentation of so-called tumor rejection antigens, and the expression of what will be referred to herein as "tumor rejection antigen precursors".

BACKGROUND AND PRIOR ART

The study of the recognition or lack of recognition of cancer cells by a host organism has proceeded in many different directions. Understanding of the field presumes some understanding of both basic immunology and oncology.

Early research on mouse tumors revealed that these displayed molecules which led to rejection of tumor cells when transplanted into syngeneic animals. These molecules are "recognized" by T-cells in the recipient animal, and provoke a cytolytic T-cell response with lysis of the transplanted cells. This evidence was first obtained with tumors induced in vitro by chemical carcinogens, such as methylcholanthrene. The antigens expressed by the tumors and which elicited the T-cell response were found to be different for each tumor. See Prehn, et al., J. Natl. Canc. Inst. 18: 769-778 (1957); Klein et al., Cancer Res. 20: 1561-1572 (1960); Gross, Cancer Res. 3: 326-333 (1943), Basombrio, Cancer Res. 30: 2458-2462 (1970) for general teachings on inducing tumors with chemical carcinogens and differences in cell surface antigens. This class of antigens has come to be known as "tumor specific transplantation antigens" or "TSTAs". Following the observation of the presentation of such antigens when induced by chemical carcinogens, similar results were obtained when tumors were induced in vitro via ultraviolet radiation. See Kripke, J. Natl. Canc. Inst. 53: 333-1336 (1974).

While T-cell mediated immune responses were observed for the types of tumor described supra, spontaneous tumors were thought to be generally non-immunogenic. These were therefore believed not to present antigens which provoked a response to the tumor in the tumor carrying subject. See Hewitt, et al., Brit. J. Cancer 33: 241-259 (1976).

The family of tum$^-$ antigen presenting cell lines are immunogenic variants obtained by mutagenesis of mouse tumor cells or cell lines, as described by Boon et al., J. Exp. Med. 152: 1184-1193 (1980), the disclosure of which is incorporated by reference. To elaborate, tum$^-$ antigens are obtained by mutating tumor cells which do not generate an immune response in syngeneic mice and will form tumors (i.e., "tum$^+$" cells). When these tum$^+$ cells are mutagenized, they are rejected by syngeneic mice, and fail to form tumors (thus "tum$^-$"). See Boon et al., Proc. Natl. Acad. Sci. USA 74: 272 (1977), the disclosure of which is incorporated by reference. Many tumor types have been shown to exhibit this phenomenon. See, e.g., Frost et al., Cancer Res. 43: 125 (1983).

It appears that tum$^-$ variants fail to form progressive tumors because they elicit an immune rejection process. The evidence in favor of this hypothesis includes the ability of "tum$^-$" variants of tumors, i.e., those which do not normally form tumors, to do so in mice with immune systems suppressed by sublethal irradiation, Van Pel et al., Proc. Natl, Acad. Sci. USA 76: 5282-5285 (1979); and the observation that intraperitoneally injected tum$^-$ cells of mastocytoma P815 multiply exponentially for 12-15 days, and then are eliminated in only a few days in the midst of an influx of lymphocytes and macrophages (Uyttenhove et al., J. Exp. Med. 152: 1175-1183 (1980)). Further evidence includes the observation that mice acquire an immune memory

which permits them to resist subsequent challenge to the same tum⁻ variant, even when immunosuppressive amounts of radiation are administered with the following challenge of cells (Boon et al., Proc. Natl, Acad. Sci. USA 74: 272-275 (1977); Van Pel et al., supra; Uyttenhove et al., supra).

Later research found that when spontaneous tumors were subjected to mutagenesis, immunogenic variants were produced which did generate a response. Indeed, these variants were able to elicit an immune protective response against the original tumor. See Van Pel et al., J. Exp. Med. 157: 1992-2001 (1983). Thus, it has been shown that it is possible to elicit presentation of a so-called "tumor rejection antigen" in a tumor which is a target for a syngeneic rejection response. Similar results have been obtained when foreign genes have been transfected into spontaneous tumors. See Fearson et al., Cancer Res. 48: 2975-1980 (1988) in this regard.

A class of antigens has been recognized which are presented on the surface of tumor cells and are recognized by cytotoxic T cells, leading to lysis. This class of antigens will be referred to as "tumor rejection antigens" or "TRAs" hereafter. TRAs may or may not elicit antibody responses. The extent to which these antigens have been studied, has been via cytolytic T cell characterization studies, in vitro i.e., the study of the identification of the antigen by a particular cytolytic T cell ("CTL" hereafter) subset. The subset proliferates upon recognition of the presented tumor rejection antigen, and

the cells presenting the antigen are lysed. Characterization studies have identified CTL clones which specifically lyse cells expressing the antigens. Examples of this work may be found in Levy et al., Adv. Cancer Res. 24: 1-59 (1977); Boon et al., J. Exp. Med. 152: 1184-1193 (1980); Brunner et al., J. Immunol. 124: 1627-1634 (1980); Maryanski et al., Eur. J. Immunol. 124: 1627-1634 (1980); Maryanski et al., Eur. J. Immunol. 12: 406-412 (1982); Palladino et al., Canc. Res. 47: 5074-5079 (1987). This type of analysis is required for other types of antigens recognized by CTLs, including minor histocompatibility antigens, the male specific H-Y antigens, and a class of antigens, referred to as "tum-" antigens, and discussed herein.

A tumor exemplary of the subject matter described supra is known as P815. See DePlaen et al., Proc. Natl. Acad. Sci. USA 85: 2274-2278 (1988); Szikora et al., EMBO J 9: 1041-1050 (1990), and Sibille et al., J. Exp. Med. 172: 35-45 (1990), the disclosures of which are incorporated by reference. The P815 tumor is a mastocytoma, induced in a DBA/2 mouse with methylcholanthrene and cultured as both an in vitro tumor and a cell line. The P815 line has generated many tum^- variants following mutagenesis, including variants referred to as P91A (DePlaen, supra), 35B (Szikora, supra), and P198 (Sibille, supra). In contrast to tumor rejection antigens - and this is a key distinction - the tum^- antigens are

only present after the tumor cells are mutagenized. Tumor rejection antigens are present on cells of a given tumor without mutagenesis. Hence, with reference to the literature, a cell line can be tum^+, such as the line referred to as "P1", and can be provoked to produce tum^- variants. Since the tum^- phenotype differs from that of the parent cell line, one expects a difference in the DNA of tum^- cell lines as compared to their tum^+ parental lines, and this difference can be exploited to locate the gene of interest in tum^- cells. As a result, it was found that genes of tum^- variants such as P91A, 35B and P198 differ from their normal alleles by point mutations in the coding regions of the gene. See Szikora and Sibille, supra, and Lurquin et al., Cell 58: 293-303 (1989). This has proved not to be the case with the TRAs of this invention. These papers also demonstrated that peptides derived from the tum^- antigen are presented by the L^d molecule for recognition by CTLs. P91A is presented by L^d, P35 by D^d and P198 by K^d.

It has now been found that the genes which code for the molecules which are processed to form the presentation tumor rejection antigens (referred to as "tumor rejection antigen precursors", "precursor molecules" or "TRAPs" hereafter), are not expressed in most normal adult tissues but are expressed in tumor cells. Genes which code for the TRAPs have now been isolated and cloned, and represent a portion of the invention disclosed herein.

The gene is useful as a source for the isolated and purified tumor rejection antigen precursor and the TRA themselves, either of which can be used as an agent for treating the cancer for which the antigen is a "marker", as well as in various diagnostic and surveillance approaches to oncology, discussed infra. It is known, for example, that tum^- cells can be used to generate CTLs which lyse cells presenting different tum^- antigens as well as tum^+ cells. See, e.g., Maryanski et al., Eur. J. Immunol 12: 401 (1982); and Van den Eynde et al., Modern Trends in Leukemia IX (June 1990), the disclosures of which are incorporated by reference. The tumor rejection antigen precursor may be expressed in cells transfected by the gene, and then used to generate an immune response against a tumor of interest.

In the parallel case of human neoplasms, it has been observed that autologous mixed lymphocyte-tumor cell cultures ("MLTC" hereafter) frequently generate responder lymphocytes which lyse autologous tumor cells and do not lyse natural killer targets, autologous EBV-transformed B cells, or autologous fibroblasts (see Anichini et al., Immunol. Today 8: 385-389 (1987)). This response has been particularly well studied for melanomas, and MLTC have been carried out either with peripheral blood cells or with tumor infiltrating lymphocytes. Examples of the literature in this area including Knuth et al., Proc. Natl. Acad. Sci. USA 86: 2804-2802 (1984); Mukherji et al., J. Exp. Med.

158: 240 (1983); Hérin et all, Int. J. Canc. 39: 390-396 (1987); Topalian et al, J. Clin. Oncol 6: 839-853 (1988). Stable cytotoxic T cell clones ("CTLs" hereafter) have been derived from MLTC responder cells, and these clones are specific for the tumor cells. See Mukherji et al., supra, Hérin et all, supra, Knuth et al., supra. The antigens recognized on tumor cells by these autologous CTLs do not appear to represent a cultural artifact, since they are found on fresh tumor cells. Topalian et al., supra; Degiovanni et al., Eur. J. Immunol. 20: 1865-1868 (1990). These observations, coupled with the techniques used herein to isolate the genes for specific murine tumor rejection antigen precursors, have led to the isolation of nucleic acid sequences coding for tumor rejection antigen precursors of TRAs presented on human tumors. It is now possible to isolate the nucleic acid sequences which code for tumor rejection antigen precursors, including, but not being limited to those most characteristic of a particular tumor, with ramifications that are described infra. These isolated nucleic acid sequences for human tumor rejection antigen precursors and applications thereof, as described infra, are also the subject of this invention.

These and various other aspects of the invention are elaborated upon in the disclosure which follows.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 depicts detection of transfectants expressing antigen P815A.

Figure 2 shows the sensitivity of clones P1.HTR, PO.HTR, genomic transfectant P1A.T2 and cosmid transfectant P1A.TC3.1 to lysis by various CTLs, as determined by chromium release assays.

Figure 3 is a restriction map of cosmid C1A.3.1.

Figure 4 shows Northern Blot analysis of expression of gene P1A.

Figure 5 sets forth the structure of gene P1A with its restriction sites.

Figure 6 shows the results obtained when cells were transfected with the gene from P1A, either isolated from P815 or normal cells and then tested with CTL lysis.

Figure 7 shows lytic studies using mast cell line L138. 8A.

Figure 8 is a map of subfragments of the 2.4 kb antigen E fragment sequence which also express the antigen.

Figure 9 shows homology of sections of exon 3 from genes mage 1, 2 and 3.

Figure 10 shows the result of Northern blots for MAGE genes on various tissues.

Figure 11 presents the data of Figure 13 in table form.

Figure 12 shows Southern Blot experiments using the various human melanoma cell lines employed in this application.

Figure 13 is a generalized schematic of the expression of MAGE 1, 2 and 3 genes by tumor and normal tissues.

BRIEF DESCRIPTION OF SEQUENCES

SEQ ID NO: 1 is cDNA for part of gene P1A.

SEQ ID NO: 2 presents coding region of cDNA for gene P1A.

SEQ ID NO: 3 shows non coding DNA for P1A cDNA which is 3′ to the coding region of SEQ ID NO: 2.

SEQ ID NO: 4 is the entire sequence of cDNA for P1A.

SEQ ID NO: 5 is the genomic DNA sequence for P1A.

SEQ ID NO: 6 shows the amino acid sequence for the antigenic peptides for P1A TRA. The sequence is for cells which are A^+ B^+, i.e., express both the A and B antigens.

SEQ ID NO: 7 is a nucleic acid sequence coding for antigen E.

SEQ ID NO: 8 is a nucleic acid sequence coding for MAGE-1.

SEQ ID NO: 9 is the gene for MAGE-2.

SEQ ID NO: 10 is the gene for MAGE-21.

SEQ ID NO: 11 is cDNA for MAGE-3.

SEQ ID NO: 12 is the gene for MAGE-31.

SEQ ID NO: 13 is the gene for MAGE-4.

SEQ ID NO: 14 is the gene for MAGE-41.

SEQ ID NO: 15 is cDNA for MAGE-4.

SEQ ID NO: 16 is cDNA for MAGE-5.

SEQ ID NO: 17 is genomic DNA for MAGE-51.

SEQ ID NO: 18 is cDNA for MAGE-6.

SEQ ID NO: 19 is genomic DNA for MAGE-7.

SEQ ID NO: 20 is genomic DNA for MAGE-8.

SEQ ID NO: 21 is genomic DNA for MAGE-9.

SEQ ID NO: 22 is genomic DNA for MAGE-10.

SEQ ID NO: 23 is genomic DNA for MAGE-11.

SEQ ID NO: 24 is genomic DNA for smage-I.

SEQ ID NO: 25 is genomic DNA for smage-II.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

Many different "MAGE" genes have been identified, as will be seen from the sequences which follow the application. The protocols described in the following

examples were used to isolate these genes and cDNA sequences.

"MAGE" as used herein refers to a nucleic acid sequence isolated from human cells. The acronym "smage" is used to describe sequences of murine origin.

When "TRAP" or "TRAs" are discussed herein as being specific to a tumor type, this means that the molecule under consideration is associated with that type of tumor, although not necessarily to the exclusion of other tumor types.

<u>Example 1</u>

In order to identify and isolate the gene coding for antigen P815A, gene transfection was used. This approach requires both a source of the gene, and a recipient cell line. Highly transfectable cell line P1.HTR was the starting material for the recipient, but it could not be used without further treatment, as it presents "antigen A", one of four recognized P815 tumor antigens. See Van Pel et al., Molecular Genetics 11: 467-475 (1985). Thus, screening experiments were carried out to isolate cell lines which did not express the antigen and which nonetheless possessed P1.HTR's desirable qualities.

To do this, P1.HTR was screened with CTLs which were specific for each of tumor antigens A, B, C and D. Such CTLs are described by Uyttenhove et al., J. Exp. Med. 157: 1040-1052 (1983).

To carry out the selection, 10^6 cells of P1.HTR were mixed with 2-4x10^6 cells of the CTL clone in a round bottom tube in 2 ml of medium, and centrifuged for three minutes at 150xg. After four hours at 37°C, the cells were washed and resuspended in 10 ml of medium, following Maryanski et al., Eur. J. Immunol. 12: 406-412 (1982). Additional information on the CTL assay and screening protocol, in general may be found in Boon et al., J. Exp. Med. 152: 1184-1193 (1980), and Maryanski et al., Eur. J. Immunol. 12: 406-412 (1982), the disclosure of which are incorporated by reference.

When these selections were carried out, a cell line variant was found which expressed neither antigen A or B. Additional selections with CTLs specific for antigen C then yielded a variant which also lacked antigen C. Please see figure 2 for a summary of the results of these screenings. The variant PO.HTR is negative for antigens A, B and C, and was therefore chosen for the transfection experiments.

The cell line PO.HTR has been deposited in accordance with the Budapest Treaty at the Institute Pasteur Collection Nationale De Cultures De Microorganismes, 28, Rue de Docteur Roux, 75724 Paris France, and has accession number I-1117.

This methodology is adaptable to secure other cell lines which are variants of a cell type which normally presents at least one of the four recognized P815 tumor antigens, i.e., antigens A, B, C and D, where the variants

present none of antigens A, B and C. P1.HTR is a mastocytoma cell line, so it will be seen that the protocol enables the isolation of biologically pure mastocytoma cell lines which express none of P815 antigens A, B and C, but which are highly transfectable. Other tumor types may also be screened in this fashion to secure desired, biologically pure cell lines. The resulting cell lines should be at least as transfectable with foreign DNA as is P1.HTR, and should be selected so as to not express a specific antigen.

Example 2

Previous work reported by DePlaen et al., Proc. Natl. Acad. Sci. USA 85: 2274-2278 (1988) the disclosure of which is incorporated by reference herein had shown the efficacy of using cosmid library transfection to recover genes coding for tum^- antigens.

Selective plasmid and genomic DNA of P1.HTR were prepared, following Wölfel et al., Immunogenetics 26: 178-187 (1987). The transfection procedure followed Corsaro et al., Somatic Cell Molec. Genet 7: 603-616 (1981), with some modification. Briefly, 60 μg of cellular DNA and 3 μg of DNA of plasmid pHMR272, described by Bernard et al., Exp. Cell. Biol. 158: 237-243 (1985) were mixed. This plasmid confers hygromycin resistance upon recipient cells, and therefore provides a convenient way to screen for transfectants. The mixed DNA was combined with 940 ul of 1 mM Tris-HCl (pH 7.5), 0.1 mM EDTA; and 310 ul 1M $CaCl_2$.

The solution was added slowly, and under constant agitation to 1.25 ml of 50 mM Hepes, 280 mM NaCl, 1.5 mM Na_2HPO_4, adjusted to pH 7.1 with NaOH. Calcium phosphate - DNA precipitates were allowed to form for 30-45 minutes at room temperature. Following this, fifteen groups of PO.HTR cells ($5x10^6$) per group were centrifuged for 10 minutes at 400 g. Supernatants were removed, and pellets were resuspended directly into the medium containing the DNA precipitates. This mixture was incubated for 20 minutes at 37°C, after which it was added to an 80 cm^2 tissue culture flask containing 22.5 ml DMEM, supplemented with 10% fetal calf serum. After 24 hours, medium was replaced. Forty-eight hours after transfection, cells were collected and counted. Transfected cells were selected in mass culture using culture medium supplemented with hygromycin B (350 ug/ml). This treatment selected cells for hygromycin resistance.

For each group, two flasks were prepared, each containing $8x10^6$ cells in 40 ml of medium. In order to estimate the number of transfectants, $1x10^6$ cells from each group were plated in 5 ml DMEM with 10% fetal calf serum (FCS), 0.4% bactoagar, and 300 ug/ml hygromycin B. The colonies were then counted 12 days later. Two independent determinations were carried out and the average taken. This was multiplied by 5 to estimate the number of transfectants in the corresponding group. Correction had

to be made for the cloning efficiency of P815 cells, known to be about 0.3.

Example 3

Eight days after transfection as described in example 2, supra, antibiotic resistant transfectants were separated from dead cells, using density centrifugation with Ficoll-Paque. These cells were maintained in non-selective medium for 1 or 2 days. The cells were plated in 96 well microplates (round bottom), at 30 cells/microwell in 200 ul of culture medium. Anywhere from 100-400 microwells were prepared, depending on the number of transfectants prepared. Agar colony tests gave estimates of 500-3000. After 5 days, the wells contained about $6x10^4$ cells and replicate plates were prepared by transferring 1/10 of the wells to microplates which were then incubated at 30°C. One day later, master plates were centrifuged, medium removed, and 750 CTLs against P815 antigen A (CTL-P1:5) were added to each well together with 10^6 irradiated syngeneic feeder spleen cells in CTL culture medium containing 40 U/ml recombinant human IL-2, and HAT medium to kill stimulator cells. Six days later, plates were examined visually to identify wells where CTLs had proliferated. Where plates showed proliferating microcultures, aliquots of 100 ul of the wells were transferred to another plate containing ^{51}Cr labeled P1.HTR target cells ($2x10^3$ - $4x10^3$ per well), and chromium release

was measured after 4 hours. Replicate microcultures corresponding to those showing high CTL activity were expanded and cloned by limited dilution in DMEM with 10% FCS. Five days later, about 200 clones were collected and screened with the CTL.P1:5 cell line, described supra, in a visual lysis assay. See figure 1A for these results.

In these experiments, three of the fifteen groups of transfectants yielded a few positive microcultures. These microcultures were tested for lytic activity against P1.HTR, as described supra. Most of the microcultures where proliferation was observed showed lytic activity. This activity was well above background, as shown in figure 1B. This figure summarizes data wherein two groups of cells (groups "5" and "14"), 400 and 300 microwells were seeded with 30 hygromycin resistant transfected cells. Amplification and duplication of the microcultures was followed by addition of anti-A CTL P1:5. Six days later, lytic activity against P1.HTR was tested. In the figure, each point represents lytic activity of a single microculture.

Duplicate microcultures corresponding to several positive wells were subcloned, and more than 1% of the subclones were found to be lysed by anti-A CTL. Thus, three independent transfectants expressing P815A were obtained from 33,000 hygromycin resistant transfectants. One of these lines, referred to hereafter as P1A.T2 was tested further.

The relevant antigen profile of P1A.T2 is shown in figure 2, this being obtained via anti-CTL assays of the type described supra.

Example 4

The CTL assays carried out for P1A.T2 demonstrated that it presented antigen A ("P815A"), and therefore had received the gene from P1.HTR. To that end, this cell line was used as a source for the gene for the antigen precursor in the following experiments.

Prior work had shown that genes coding for tum$^-$ antigens could be recovered directly from transfectants obtained with a cosmid library. See DePlaen et al., Proc. Natl. Acad. Sci. USA 85: 2274-2278 (1988). This procedure was followed for recovery of the P815 gene.

Total genomic DNA of P1A.T2 was partially digested with restriction endonuclease Sau 3A1, and fractionated by NaCl density gradient ultracentrifugation to enrich for 35-50 kb DNA fragments, following Grosveld et al., Gene 10: 6715-6732 (1982). These fragments were ligated to cosmid arms of C2RB, described by Bates et al., Gene 26: 137-146 (1983), the disclosure of which is incorporated by reference. These cosmid arms had been obtained by cleavage with SmaI and treatment with calf intestinal phosphatase, followed by digestion with BamHI. Ligated DNA was packaged into lambda phage components, and titrated on E. coli ED 8767, following Grosveld et al., supra. Approximately 9×10^5

ampicillin resistant colonies were obtained per microgram of DNA insert.

The cosmid groups were amplified by mixing 30,000 independent cosmids with 2 ml of ED 8767 in 10 mM $MgCl_2$, incubated 20 minutes at 37°C, diluted with 20 ml of Luria Bertani ("LB") medium, followed by incubation for one hour. This suspension was titrated and used to inoculate 1 liter of LB medium in the presence of ampicillin (50 ug/ml). At a bacterial concentration of $2x10^8$ cells/ml (OD_{600}=0.8), a 10 ml aliquot was frozen, and 200 ug/ml chloramphenicol was added to the culture for overnight incubation. Total cosmid DNA was isolated by alkaline lysis procedure, and purified on CsCl gradient.

In these experiments, a library of 650,000 cosmids was prepared. The amplification protocol involved the use of 21 groups of approximately 30,000 cosmids.

Example 5

Using the twenty-one groups of cosmids alluded to *supra*, (60 ug) and 4 ug of pHMR272, described *supra*, groups of $5x10^6$ PO.HTR cells were used as transfectant hosts. Transfection was carried out in the same manner as described in the preceding experiments. An average of 3000 transfectants per group were tested for antigen presentation, again using CTL assays as described. One group of cosmids repeatedly yielded positive transfectants, at a frequency of about 1/5,000 drug resistant

transfectants. The transfectants, as with P1A.T2, also showed expression of both antigen A and B. The pattern of expression of transfectant P1A.TC3.1 is shown in figure 2.

Example 6

As indicated in Example 5, supra, three independent cosmid transfected cells presenting P815A antigen were isolated. The DNA of these transfectants was isolated and packaged directly with lambda phage extracts, following DePlaen et al., Proc. Natl. Acad. Sci. USA 85: 2274-2278 (1988). The resulting product was titrated on E. coli ED 8767 with ampicillin selection, as in Example 5. Similarly, amplification of the cosmids and transfection followed Example 5, again using PO.HTR.

High frequencies of transfection were observed, as described in Table 1, which follows:

Table 1. Transfer of the expression of antigen P815A by cosmids obtained by direct packaging

Transfectant obtained with the cosmid library	No. of cosmids obtained by direct packaging of 0.5 µg of DNA	No. of transfectants expressing P815A / no. of HmB^1 transfectants
TC3.1	32	87/192
TC3.2	32000	49/384
TC3.3	44	25/72

The cosmids were analyzed with restriction enzymes and it was found that directly packaged transfectant P1A.TC3.1 contained 32 cosmids, 7 of which were different. Each of these 7 cosmids was transfected into PO.HTR, in the manner described supra, and again, following the protocols described above, transfectants were studied for presentation of P815A. Four of the cosmid transfectants showed P815A presentation and, as with all experiments described herein, P815B was co-expressed.

Of the four cosmids showing presentation of the two antigens, cosmid C1A.3.1 was only 16.7 kilobases long, and was selected for further analysis as described infra.

The cosmid C1A.3.1 was subjected to restriction endonuclease analysis, yielding the map shown in Figure 3.

All EcoRI fragments were transfected, again using the above described protocols, and only the 7.4 kilobase fragment produced a transfectant that anti-A CTLs could lyse. Similar experiments were carried out on the PstI fragments, and only a 4.1 kb fragment fully contained within the 7.4 kb EcoRI fragment produced lysable transfectants.

This fragment (i.e., the 4.1 kb PstI fragment), was digested with SmaI, giving a 2.3 kb fragment which also yielded host cells presenting antigens A and B after transfection. Finally, a fragment 900 bases long, secured with SmaI/XbaI, also transferred expression of the precursors of these two antigens, i.e., the transfected host cell presented both antigen A and antigen B.

Example 7

The 900 base fragment described above was used as a probe to detect the expression of the P815A gene in parent cell line P1.HTR. To accomplish this, total cellular RNA was first isolated using the guanidine-isothiocyanate procedure of Davis et al., Basic Methods In Molecular Biology (Elseview Science Publishing Co, New York) (1986). The same reference was the source of the method used to isolate and purify polyA$^+$ mRNA using oligodT cellulose column chromatography.

Samples were then subjected to Northern Blot analysis. RNA samples were fractionated on 1% agarose gels containing 0.66 M formaldehyde. The gels were treated with 10xSSC (SSC: 0.15 M NaCl; 0.015 M sodium citrate, pH 7.0) for 30 minutes before overnight blotting on nitrocellulose membranes. These were baked for two hours at 80°C, after which the membranes were prehybridized for 15 minutes at 60°C in a solution containing 10% dextran sulfate, 1% SDS and 1M NaCl. Hybridization was then carried out using denatured probe (the 900 base fragment), together with 100 ug/ml salmon sperm DNA.

When this protocol was carried out using P1.HTR poly A$^+$ RNA, a band of 1.2 kb and two fainter bands were identified, as shown in Figure 4, lane 1 (6 ug of the RNA).

The same probe was used to screen a cDNA library, prepared from poly-A$^+$ RNA from the cell line. This yielded

a clone with a 1kb insert, suggesting a missing 5′ end. The Northern blots for the cDNA are not shown.

Hybridization experiments in each case were carried out overnight at 60°C. The blots were washed twice at room temperature with 2xSSC and twice at 60°C with 2xSSC supplemented with 1% SDS.

The foregoing experiments delineated the DNA expressing the P815A antigen precursor sufficiently to allow sequencing, using the well known Sanger dideoxy chain termination method. This was carried out on clones generated using a variety of restriction endonucleases and by specific priming with synthetic oligonucleotide primers. The results for exons of the gene are set forth in sequence id no: 4.

Example 8

The Northern analysis described supra suggested that the 5′ end of the cDNA was missing. To obtain this sequence, cDNA was prepared from P1.HTR RNA using a primer corresponding to positions 320-303. The sequence was then amplified using the polymerase chain reaction using a 3′ primer corresponding to positions 286-266 and a 5′ primer described by Frohman et al., Proc. Natl. Acad. Sci. USA 85: 8998-9002 (1988). A band of the expected size (270 bases) was found, which hybridized to the 900 bp SmaI/XbaI fragment described supra on a Southern blot. Following cloning into m13tg 130 λ tg 131, the small, 270 bp fragment was sequenced. The sequence is shown in sequence id no: 1.

Example 9

Following the procurement of the sequences described in Examples 7 and 8 and depicted in seq id no: 4, a 5.7 kb region of cosmid C1A.3.1 was sequenced. This fragment was known to contain the 900 base fragment which expressed P815A in transfectants. This experiment permitted delineation of introns and exons, since the cosmid is genomic in origin.

The delineated structure of the gene is shown in figure 5. Together with seq id no: 4, these data show that the gene for the antigen precursor, referred to as "P1A" hereafter, is approximately 5 kilobases long and contains 3 exons. An ORF for a protein of 224 amino acids starts in exon 1, ending in exon 2. The 900 base pair fragment which transfers expression of precursors for antigens A and B only contains exon 1. The promoter region contains a CAAT box, as indicated in seq. id no: 1, and an enhancer sequence. This latter feature has been observed in promoters of most MHC class I genes, as observed by Geraghty et al., J. Exp. Med 171: 1-18 (1990); Kimura et al., Cell 44: 261-272 (1986).

A computer homology search was carried out, using program FASTA with K-triple parameters of 3 and 6, as suggested by Lipman et al., Science 227: 1435-1441 (1985), and using Genbank database release 65 (October 1990). No homology was found except for a stretch of 95 bases corresponding to part of an acid region coded by exon 1 (positions 524-618), which is similar to sequences coding

for acidic regions in mouse nucleolar protein NO38/B23, as described by Bourbon et al., Mol. Biol. 200: 627-638 (1988), and Schmidt-Zachmann et al., Chromosoma 96: 417-426 (1988). Fifty six of 95 bases were identical. In order to test whether these homologies were the reason for cross hybridizing, experiments were carried out using a mouse spleen cDNA library screened with the 900 base fragment. cDNA clones corresponding closely to the sizes of the cross hybridizing bands were obtained. These were partially sequenced, and the 2.6 kb cDNA was found to correspond exactly to reported cDNA sequence of mouse nucleolin, while the 1.5 kb cDNA corresponded to mouse nucleolar protein NO38/B23.

Analysis of the nucleotide sequence of the gene, referred to as "P1A" hereafter, suggests that its coded product has a molecular mass of 25 kd. Analysis of the sequence id no: 4 shows a potential nuclear targeting signal at residues 5-9 (Dingwall et al., Ann. Rev. Cell Biol. 2: 367-390 (1986)), as well as a large acidic domain at positions 83-118. As indicated supra, this contains the region of homology between P1A and the two nucleolar proteins. A putative phosphorylation site can be found at position 125 (serine). Also, a second acidic domain is found close to the C-terminus as an uninterrupted stretch of 14 glutamate residues. A similar C-terminal structure has been found by Kessel et al. Proc. Natl. Acad. Sci. USA 84: 5306-5310 (1987), in a murine homeodomain protein having nuclear localization.

In studies comparing the sequence of gene P1A to the sequences for P91A, 35B and P198, no similarities were found, showing that P1A is indicative of a different class of genes and antigens.

Example 10

With the P1A probe and sequence in hand, investigations were carried out to determine whether the gene present in normal tissue was identical to that expressed by the tumor. To do this, phage libraries were prepared, using lambda zapII 10 and genomic DNA of DBA2 murine kidney cells. P1A was used as a probe. Hybridization conditions were as described supra, and a hybridizing clone was found. The clone contained exons one and two of the P1A gene, and corresponded to positions -0.7 to 3.8 of figure 5. Following localization of this sequence, PCR amplification was carried out to obtain the sequence corresponding to 3.8 to 4.5 of figure 5.

Sequence analysis was carried out, and no differences were found between the gene from normal kidneys and the P1A gene as obtained from the P815 tumor cells.

In further experiments, the gene as found in DBA/2 kidney cells was transfected into PO.HTR, as described supra. These experiments, presented pictorially in figure 7, showed that antigens A and B were expressed as efficiently by the kidney gene isolated from normal kidney cells as with the P1A gene isolated from normal kidney cells.

These experiments lead to the conclusion that the gene coding for the tumor rejection antigen precursor is a gene that does not result from a mutation; rather, it would appear that the gene is the same as one present in normal cells, but is not expressed therein. The ramifications of this finding are important, and are discussed infra.

In studies not elaborated upon herein, it was found that variants of the gene were available. Some cells were "P1A^{-}B^{+}", rather than the normal "P1A". The only difference between these is a point mutation in exon 1, with the 18th triplet coding for Ala in the variant instead of Val.

Example 11

Additional experiments were carried out with other cell types. Following the protocols described for Northern blot hybridizations supra, RNA of normal liver and spleen cells was tested to determine if a transcript of the P1A gene could be found. The Northern blot data are presented in figure 4 and, as can be seen, there is no evidence of expression.

The murine P815 cell line from which P1A was isolated is a mastocytoma. Therefore, mast cell lines were studied to determine if they expressed the gene. Mast cell line MC/9, described by Nabel et al., Cell 23: 19-28 (1981), and short term cultures of bone marrow derived mast cells were tested in the manner described supra (Northern blotting), but no transcript was found. In contrast when a Balb/C derived IL-3 dependent cell line L138.8A (Hültner et al.,

J. Immunol. 142: 3440-3446 (1989)) was tested, a strong signal was found. The mast cell work is shown in figure 4.

It is known that both BALB/C and DBA/2 mice share $H\text{-}2^d$ haplotype, and thus it was possible to test sensitivity to lysis using the CTLs described supra. Figure 8 shows these results, which essentially prove that anti-A and anti-B CTLs lysed the cells strongly, whereas anti-C and anti-D lines did not.

Further tests were carried out on other murine tumor cell lines, i.e., teratocarcinoma cell line PCC4 (Boon et al., Proc. Natl. Acad. Sci. USA 74: 272-275 (1977), and leukemias LEC and WEH1-3B. Expression could not be detected in any of these samples.

Example 12

The actual presentation of the P1A antigen by MHC molecules was of interest. To test this, cosmid C1A.3.1 was transfected into fibroblast cell line DAP, which shows phenotype $H\text{-}2^k$. The cell lines were transfected with genes expressing one of the K^d, D^d, and L^d antigen. Following transfection with both the cosmid and the MHC gene, lysis with CTLs was studied, again as described supra. These studies, summarized in Table 2, show that L^d is required for presentation of the P1A antigens A and B.

Table 2. H-2-restriction of antigens P815A and P815B

Recipient cell*	No of clones lysed by the CTL/ no. of $Hm^{r}B^{r}$ clones*	
	CTL anti-A	CTL anti-B
DAP ($H\text{-}2^{k}$)	0/208	0/194
DAP + K^{d}	0/165	0/162
DAP + D^{d}	0/157	0/129
DAP + L^{d}	25/33	15/20

*Cosmid C1A.3.1 containing the entire P1A gene was transfected in DAP cells previously transfected with $H\text{-}2^{d}$ class I genes as indicated.

*Independent drug-resistant colonies were tested for lysis by anti-A or anti-B CTL in a visual assay.

The observation that one may associate presentation of a tumor rejection antigen with a particular MHC molecule was confirmed in experiments with human cells and HLA molecules, as elaborated upon infra.

Example 13

Using the sequence of the P1A gene as well as the amino acid sequence derivable therefrom, antigenic peptides which were A^{+} B^{+} (i.e., characteristic of cells which express both the A and B antigens), and those which are A^{-} B^{+} were identified. The peptide is presented in Figure 10. This peptide when administered to samples of PO.HTR cells

in the presence of CTL cell lines specific to cells presenting it, led to lysis of the PO.HTR cells, lending support to the view that peptides based on the product expressed by the gene can be used as vaccines.

Example 14

The human melanoma cell line referred to hereafter as MZ2-MEL is not a clonal cell line. It expresses four stable antigens recognized by autologous CTLs, known as antigens "D, E, F, and A". In addition, two other antigens "B" and "C" are expressed by some sublines of the tumor. CTL clones specific for these six antigens are described by Van den Eynde et al., Int. J. Canc. 44: 634-640 (1989). Among the recognized subclones of MZ2-MEL are MEL.43, MEL3.0 and MEL3.1. (Van den Eynde et al., supra). Cell line MEL3.1 expresses antigen E, as determined by CTL studies as described for P815 variants, supra, so it was chosen as a source for the nucleic acid sequence expressing the antigen precursor.

In isolating the pertinent nucleic acid sequence for a tumor rejection antigen precursor, the techniques developed supra, showed that a recipient cell is needed which fulfills two criteria: (i) the recipient cell must not express the TRAP of interest under normal conditions, and (ii) it must express the relevant class I HLA molecule. Also, the recipient cell must have a high transfection frequency, i.e., it must be a "good" recipient.

In order to secure such a cell line, the clonal subline ME3.1 was subjected to repeated selection with anti-E CTL 82/30 as described by Van den Eynde, supra. The repeated cycles of selection led to isolation of subclone MZ2-MEL-2.2 isc E^-. This subclone is also $HPRT^-$, (i.e., sensitive to HAT medium: 10^{-4} M hypoxanthine, 3.8×10^{-7} aminopterine, 1.6×10^{-5} M 2-deoxythymidine). The subclone is referred to as "MEL-2.2" for simplicity hereafter.

Example 15

The genomic DNA of MEL3.0 was prepared following Wölfel et al., Immunogenetics 26: 178-187 (1987), the disclosure of which is incorporated by reference. The plasmid pSVtkneoß, as described by Nicolas et al., Cold Spring Harb., Conf. Cell Prolif. 10: 469-485 (1983) confers geneticin resistance, so it can be used as a marker for cotransfection, as it was in this experiment.

Following a procedure similar but not identical to that of Corsao et al., Somatic Cell Molec. Genet 7: 603-616 (1981), total genomic DNA and the plasmid were cotransfected. The genomic DNA (60 µg) and plasmid DNA (6 µg) were mixed in 940 µl of 1 mM Tris·HCl (pH 7.5), 0.1 mM EDTA, after which 310 µl of 1M $CaCl_2$ was added. This solution was slowly added, under constant agitation, to 1.25 ml of 2xHBS (50 mM HEPES, 280 mM NaCl 1.5 mM Na_2HPO_4, adjusted to pH 7.1 with NaOH). The calcium phosphate DNA precipitates were allowed to form for 30-45 minutes at room

temperature, after which they were applied to 80 cm^2 tissue culture flasks which had been seeded 24 hours previously with $3x10^6$ MEL2.2 cells, in 22.5 ml of melanoma culture medium (Dulbecco's Modified Eagle's Medium) supplemented with 10% fetal calf serum. After 24 hours, the medium was replaced. Forty eight hours after transfection, the cells were harvested and seeded at $4x10^6$ cells per 80 cm^2 flask in melanoma culture medium supplemented with 2 mg/ml of geneticin. The geneticin serves as a selection marker.

Example 16

Thirteen days after transfection, geneticin-resistant colonies were counted, harvested, and cultured in nonselective medium for 2 or 3 days. Transfected cells were then plated in 96-well microplates at 200 cells/well in 200 ul of culture medium with 20% fetal calf serum (FCS) in order to obtain approximately 30 growing colonies per well. The number of microcultures was aimed at achieving redundancy, i.e., such that every independent transfectant should be represented at least four times.

After 10 days, wells contained approximately $6x10^4$ cells. These cells were detached, and 1/3 of each microculture was transferred to a duplicate plate. After 6 hours, i.e., after readherence, medium was removed and 1500 anti-E CTL (CTL 82/30), were added to each well in 100 μl of CTL culture medium with 35 U/ml of IL-2. One day later, the supernatant (50 μl) was harvested and examined

for TNF concentration, for reasons set forth in the following example.

Example 17

The size of the mammalian genome is $6x10^6$ kb. As the average amount of DNA integrated in each drug-resistant transfectant was expected to be about 200 kb, a minimum of 30,000 transfectants would need to be examined to ascertain whether antigen E had been transfected. Prior work with murine cells had shown that when a CTL stimulation assay was used, groups containing only 3% of cells expressing the antigen of interested could be identified. This should reduce the number of assays by a factor of 30. While an anti-E CTL assay, as described supra, in mixed E^+/E^- cells was helpful, it was not sufficient in that consistent results could not be obtained.

As a result, an alternative test was devised. Stimulation of CTLs was studied by release of tumor necrosis factor ("TNF") using well known methodologies which need not be repeated here. As described in Example 15, 1500 CTL 82/30 cells had been added per well of transfectants. These CTLs were collected 6 days after stimulation. As indicated supra, after 1/3 of the cells in each well had been removed and the remaining 2/3 ($4x10^4$) had readhered, the CTLs and IL-2 were added thereto. The 50 μl of supernatant was removed 24 hours later and transferred to a microplate containing $3x10^4$ W13 (WEHI-164 clone 13;

Espevik et al., J. Immunol. Meth. 95: 99-105 (1986)) cells in 50 μl of W13 culture medium (RPMI-1640, supplemented with L-arginine (116 mg/l), L-asparagine (36 mg/l), L-glutamine (216 mg/l), and 10% FCS supplemented with 2 μg of actinomycin D at 37% in an 8% CO_2 atmosphere. The cell line W13 is a mouse fibrosarcoma cell line sensitive to TNF. Dilutions of recombinant TNF-β in RPMI 1640 were added to target cell controls.

The W13 cultures were evaluated after 20 hours of incubation, and dead cell percentage was measured using an adaptation of the colorimetric assay of Hansen et al., J. Immunol. Meth. 119: 203-210 (1989). This involved adding 50 ml of (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide at 2.5 mg/ml in PBS, followed by two hours of incubation at 37°C. Dark blue formazan crystals were dissolved by adding 100 μl of lysis solution (1 volume N,N dimethyl formamide mixed at 37°C with two volumes of water containing 30% (w/v) sodium dodecyl sulphate, at pH 4.7 from 1.6% acetic acid and 2.5% 1N HCl). Plates were incubated at 37°C overnight, and ODs were taken at 570 nm using 650 nm as control. Dead cell percentage was determined via the formula:

$$100 \times \left[1 - \frac{100-(OD_{570} \text{ sample well})}{OD_{570} \text{ well + medium}} \right]$$

To carry out the selection, 10^6 cells of P1.HTR were mixed with 2-4x10^6 cells of the CTL clone in a round bottom tube in 2 ml of medium, and centrifuged for three minutes at 150xg. After four hours at 37°C, the cells were washed and resuspended in 10 ml of medium, following Maryanski et al., Eur. J. Immunol. 12: 406-412 (1982). Additional information on the CTL assay and screening protocol, in general may be found in Boon et al., J. Exp. Med. 152: 1184-1193 (1980), and Maryanski et al., Eur. J. Immunol. 12: 406-412 (1982), the disclosure of which are incorporated by reference.

When these selections were carried out, a cell line variant was found which expressed neither antigen A or B. Additional selections with CTLs specific for antigen C then yielded a variant which also lacked antigen C. Please see figure 2 for a summary of the results of these screenings. The variant PO.HTR is negative for antigens A, B and C, and was therefore chosen for the transfection experiments.

The cell line PO.HTR has been deposited in accordance with the Budapest Treaty at the Institute Pasteur Collection Nationale De Cultures De Microorganismes, 28, Rue de Docteur Roux, 75724 Paris France, and has accession number I-1117.

This methodology is adaptable to secure other cell lines which are variants of a cell type which normally presents at least one of the four recognized P815 tumor antigens, i.e., antigens A, B, C and D, where the variants

present none of antigens A, B and C. P1.HTR is a mastocytoma cell line, so it will be seen that the protocol enables the isolation of biologically pure mastocytoma cell lines which express none of P815 antigens A, B and C, but which are highly transfectable. Other tumor types may also be screened in this fashion to secure desired, biologically pure cell lines. The resulting cell lines should be at least as transfectable with foreign DNA as is P1.HTR, and should be selected so as to not express a specific antigen.

Example 2

Previous work reported by DePlaen et al., Proc. Natl. Acad. Sci. USA 85: 2274-2278 (1988) the disclosure of which is incorporated by reference herein had shown the efficacy of using cosmid library transfection to recover genes coding for tum$^-$ antigens.

Selective plasmid and genomic DNA of P1.HTR were prepared, following Wölfel et al., Immunogenetics 26: 178-187 (1987). The transfection procedure followed Corsaro et al., Somatic Cell Molec. Genet 7: 603-616 (1981), with some modification. Briefly, 60 μg of cellular DNA and 3 μg of DNA of plasmid pHMR272, described by Bernard et al., Exp. Cell. Biol. 158: 237-243 (1985) were mixed. This plasmid confers hygromycin resistance upon recipient cells, and therefore provides a convenient way to screen for transfectants. The mixed DNA was combined with 940 ul of 1 mM Tris-HCl (pH 7.5), 0.1 mM EDTA; and 310 ul 1M $CaCl_2$.

The solution was added slowly, and under constant agitation to 1.25 ml of 50 mM Hepes, 280 mM NaCl, 1.5 mM Na_2HPO_4, adjusted to pH 7.1 with NaOH. Calcium phosphate - DNA precipitates were allowed to form for 30-45 minutes at room temperature. Following this, fifteen groups of PO.HTR cells ($5x10^6$) per group were centrifuged for 10 minutes at 400 g. Supernatants were removed, and pellets were resuspended directly into the medium containing the DNA precipitates. This mixture was incubated for 20 minutes at 37°C, after which it was added to an 80 cm^2 tissue culture flask containing 22.5 ml DMEM, supplemented with 10% fetal calf serum. After 24 hours, medium was replaced. Forty-eight hours after transfection, cells were collected and counted. Transfected cells were selected in mass culture using culture medium supplemented with hygromycin B (350 ug/ml). This treatment selected cells for hygromycin resistance.

For each group, two flasks were prepared, each containing $8x10^6$ cells in 40 ml of medium. In order to estimate the number of transfectants, $1x10^6$ cells from each group were plated in 5 ml DMEM with 10% fetal calf serum (FCS), 0.4% bactoagar, and 300 ug/ml hygromycin B. The colonies were then counted 12 days later. Two independent determinations were carried out and the average taken. This was multiplied by 5 to estimate the number of transfectants in the corresponding group. Correction had

to be made for the cloning efficiency of P815 cells, known to be about 0.3.

Example 3

Eight days after transfection as described in example 2, supra, antibiotic resistant transfectants were separated from dead cells, using density centrifugation with Ficoll-Paque. These cells were maintained in non-selective medium for 1 or 2 days. The cells were plated in 96 well microplates (round bottom), at 30 cells/microwell in 200 ul of culture medium. Anywhere from 100-400 microwells were prepared, depending on the number of transfectants prepared. Agar colony tests gave estimates of 500-3000. After 5 days, the wells contained about $6x10^4$ cells and replicate plates were prepared by transferring 1/10 of the wells to microplates which were then incubated at 30°C. One day later, master plates were centrifuged, medium removed, and 750 CTLs against P815 antigen A (CTL-P1:5) were added to each well together with 10^6 irradiated syngeneic feeder spleen cells in CTL culture medium containing 40 U/ml recombinant human IL-2, and HAT medium to kill stimulator cells. Six days later, plates were examined visually to identify wells where CTLs had proliferated. Where plates showed proliferating microcultures, aliquots of 100 ul of the wells were transferred to another plate containing ^{51}Cr labeled P1.HTR target cells ($2x10^3$ - $4x10^3$ per well), and chromium release

was measured after 4 hours. Replicate microcultures corresponding to those showing high CTL activity were expanded and cloned by limited dilution in DMEM with 10% FCS. Five days later, about 200 clones were collected and screened with the CTL.P1:5 cell line, described supra, in a visual lysis assay. See figure 1A for these results.

In these experiments, three of the fifteen groups of transfectants yielded a few positive microcultures. These microcultures were tested for lytic activity against P1.HTR, as described supra. Most of the microcultures where proliferation was observed showed lytic activity. This activity was well above background, as shown in figure 1B. This figure summarizes data wherein two groups of cells (groups "5" and "14"), 400 and 300 microwells were seeded with 30 hygromycin resistant transfected cells. Amplification and duplication of the microcultures was followed by addition of anti-A CTL P1:5. Six days later, lytic activity against P1.HTR was tested. In the figure, each point represents lytic activity of a single microculture.

Duplicate microcultures corresponding to several positive wells were subcloned, and more than 1% of the subclones were found to be lysed by anti-A CTL. Thus, three independent transfectants expressing P815A were obtained from 33,000 hygromycin resistant transfectants. One of these lines, referred to hereafter as P1A.T2 was tested further.

The relevant antigen profile of P1A.T2 is shown in figure 2, this being obtained via anti-CTL assays of the type described supra.

Example 4

The CTL assays carried out for P1A.T2 demonstrated that it presented antigen A ("P815A"), and therefore had received the gene from P1.HTR. To that end, this cell line was used as a source for the gene for the antigen precursor in the following experiments.

Prior work had shown that genes coding for tum$^-$ antigens could be recovered directly from transfectants obtained with a cosmid library. See DePlaen et al., Proc. Natl. Acad. Sci. USA 85: 2274-2278 (1988). This procedure was followed for recovery of the P815 gene.

Total genomic DNA of P1A.T2 was partially digested with restriction endonuclease Sau 3A1, and fractionated by NaCl density gradient ultracentrifugation to enrich for 35-50 kb DNA fragments, following Grosveld et al., Gene 10: 6715-6732 (1982). These fragments were ligated to cosmid arms of C2RB, described by Bates et al., Gene 26: 137-146 (1983), the disclosure of which is incorporated by reference. These cosmid arms had been obtained by cleavage with SmaI and treatment with calf intestinal phosphatase, followed by digestion with BamHI. Ligated DNA was packaged into lambda phage components, and titrated on E. coli ED 8767, following Grosveld et al., supra. Approximately $9x10^5$

ampicillin resistant colonies were obtained per microgram of DNA insert.

The cosmid groups were amplified by mixing 30,000 independent cosmids with 2 ml of ED 8767 in 10 mM $MgCl_2$, incubated 20 minutes at 37°C, diluted with 20 ml of Luria Bertani ("LB") medium, followed by incubation for one hour. This suspension was titrated and used to inoculate 1 liter of LB medium in the presence of ampicillin (50 ug/ml). At a bacterial concentration of $2x10^8$ cells/ml (OD_{600}=0.8), a 10 ml aliquot was frozen, and 200 ug/ml chloramphenicol was added to the culture for overnight incubation. Total cosmid DNA was isolated by alkaline lysis procedure, and purified on CsCl gradient.

In these experiments, a library of 650,000 cosmids was prepared. The amplification protocol involved the use of 21 groups of approximately 30,000 cosmids.

Example 5

Using the twenty-one groups of cosmids alluded to supra, (60 ug) and 4 ug of pHMR272, described supra, groups of $5x10^6$ PO.HTR cells were used as transfectant hosts. Transfection was carried out in the same manner as described in the preceding experiments. An average of 3000 transfectants per group were tested for antigen presentation, again using CTL assays as described. One group of cosmids repeatedly yielded positive transfectants, at a frequency of about 1/5,000 drug resistant

transfectants. The transfectants, as with P1A.T2, also showed expression of both antigen A and B. The pattern of expression of transfectant P1A.TC3.1 is shown in figure 2.

Example 6

As indicated in Example 5, supra, three independent cosmid transfected cells presenting P815A antigen were isolated. The DNA of these transfectants was isolated and packaged directly with lambda phage extracts, following DePlaen et al., Proc. Natl. Acad. Sci. USA 85: 2274-2278 (1988). The resulting product was titrated on E. coli ED 8767 with ampicillin selection, as in Example 5. Similarly, amplification of the cosmids and transfection followed Example 5, again using PO.HTR.

High frequencies of transfection were observed, as described in Table 1, which follows:

Table 1. Transfer of the expression of antigen P815A by cosmids obtained by direct packaging

Transfectant obtained with the cosmid library	No. of cosmids obtained by direct packaging of 0.5 µg of DNA	No. of transfectants expressing P815A / no. of $H-2^d$ transfectants
TC3.1	32	87/192
TC3.2	32000	49/384
TC3.3	44	25/72

The cosmids were analyzed with restriction enzymes and it was found that directly packaged transfectant P1A.TC3.1 contained 32 cosmids, 7 of which were different. Each of these 7 cosmids was transfected into PO.HTR, in the manner described supra, and again, following the protocols described above, transfectants were studied for presentation of P815A. Four of the cosmid transfectants showed P815A presentation and, as with all experiments described herein, P815B was co-expressed.

Of the four cosmids showing presentation of the two antigens, cosmid C1A.3.1 was only 16.7 kilobases long, and was selected for further analysis as described infra.

The cosmid C1A.3.1 was subjected to restriction endonuclease analysis, yielding the map shown in Figure 3.

All EcoRI fragments were transfected, again using the above described protocols, and only the 7.4 kilobase fragment produced a transfectant that anti-A CTLs could lyse. Similar experiments were carried out on the PstI fragments, and only a 4.1 kb fragment fully contained within the 7.4 kb EcoRI fragment produced lysable transfectants.

This fragment (i.e., the 4.1 kb PstI fragment), was digested with SmaI, giving a 2.3 kb fragment which also yielded host cells presenting antigens A and B after transfection. Finally, a fragment 900 bases long, secured with SmaI/XbaI, also transferred expression of the precursors of these two antigens, i.e., the transfected host cell presented both antigen A and antigen B.

Example 7

The 900 base fragment described above was used as a probe to detect the expression of the P815A gene in parent cell line P1.HTR. To accomplish this, total cellular RNA was first isolated using the guanidine-isothiocyanate procedure of Davis et al., Basic Methods In Molecular Biology (Elseview Science Publishing Co, New York) (1986). The same reference was the source of the method used to isolate and purify polyA$^+$ mRNA using oligodT cellulose column chromatography.

Samples were then subjected to Northern Blot analysis. RNA samples were fractionated on 1% agarose gels containing 0.66 M formaldehyde. The gels were treated with 10xSSC (SSC: 0.15 M NaCl; 0.015 M sodium citrate, pH 7.0) for 30 minutes before overnight blotting on nitrocellulose membranes. These were baked for two hours at 80°C, after which the membranes were prehybridized for 15 minutes at 60°C in a solution containing 10% dextran sulfate, 1% SDS and 1M NaCl. Hybridization was then carried out using denatured probe (the 900 base fragment), together with 100 ug/ml salmon sperm DNA.

When this protocol was carried out using P1.HTR poly A$^+$ RNA, a band of 1.2 kb and two fainter bands were identified, as shown in Figure 4, lane 1 (6 ug of the RNA).

The same probe was used to screen a cDNA library, prepared from poly-A$^+$ RNA from the cell line. This yielded

a clone with a 1kb insert, suggesting a missing 5′ end. The Northern blots for the cDNA are not shown.

Hybridization experiments in each case were carried out overnight at 60°C. The blots were washed twice at room temperature with 2xSSC and twice at 60°C with 2xSSC supplemented with 1% SDS.

The foregoing experiments delineated the DNA expressing the P815A antigen precursor sufficiently to allow sequencing, using the well known Sanger dideoxy chain termination method. This was carried out on clones generated using a variety of restriction endonucleases and by specific priming with synthetic oligonucleotide primers. The results for exons of the gene are set forth in sequence id no: 4.

Example 8

The Northern analysis described supra suggested that the 5′ end of the cDNA was missing. To obtain this sequence, cDNA was prepared from P1.HTR RNA using a primer corresponding to positions 320-303. The sequence was then amplified using the polymerase chain reaction using a 3′ primer corresponding to positions 286-266 and a 5′ primer described by Frohman et al., Proc. Natl. Acad. Sci. USA 85: 8998-9002 (1988). A band of the expected size (270 bases) was found, which hybridized to the 900 bp SmaI/XbaI fragment described supra on a Southern blot. Following cloning into m13tg 130 λ tg 131, the small, 270 bp fragment was sequenced. The sequence is shown in sequence id no: 1.

Example 9

Following the procurement of the sequences described in Examples 7 and 8 and depicted in seq id no: 4, a 5.7 kb region of cosmid C1A.3.1 was sequenced. This fragment was known to contain the 900 base fragment which expressed P815A in transfectants. This experiment permitted delineation of introns and exons, since the cosmid is genomic in origin.

The delineated structure of the gene is shown in figure 5. Together with seq id no: 4, these data show that the gene for the antigen precursor, referred to as "P1A" hereafter, is approximately 5 kilobases long and contains 3 exons. An ORF for a protein of 224 amino acids starts in exon 1, ending in exon 2. The 900 base pair fragment which transfers expression of precursors for antigens A and B only contains exon 1. The promoter region contains a CAAT box, as indicated in seq. id no: 1, and an enhancer sequence. This latter feature has been observed in promoters of most MHC class I genes, as observed by Geraghty et al., J. Exp. Med 171: 1-18 (1990); Kimura et al., Cell 44: 261-272 (1986).

A computer homology search was carried out, using program FASTA with K-triple parameters of 3 and 6, as suggested by Lipman et al., Science 227: 1435-1441 (1985), and using Genbank database release 65 (October 1990). No homology was found except for a stretch of 95 bases corresponding to part of an acid region coded by exon 1 (positions 524-618), which is similar to sequences coding

for acidic regions in mouse nucleolar protein NO38/B23, as described by Bourbon et al., Mol. Biol. 200: 627-638 (1988), and Schmidt-Zachmann et al., Chromosoma 96: 417-426 (1988). Fifty six of 95 bases were identical. In order to test whether these homologies were the reason for cross hybridizing, experiments were carried out using a mouse spleen cDNA library screened with the 900 base fragment. cDNA clones corresponding closely to the sizes of the cross hybridizing bands were obtained. These were partially sequenced, and the 2.6 kb cDNA was found to correspond exactly to reported cDNA sequence of mouse nucleolin, while the 1.5 kb cDNA corresponded to mouse nucleolar protein NO38/B23.

Analysis of the nucleotide sequence of the gene, referred to as "P1A" hereafter, suggests that its coded product has a molecular mass of 25 kd. Analysis of the sequence id no: 4 shows a potential nuclear targeting signal at residues 5-9 (Dingwall et al., Ann. Rev. Cell Biol. 2: 367-390 (1986)), as well as a large acidic domain at positions 83-118. As indicated supra, this contains the region of homology between P1A and the two nucleolar proteins. A putative phosphorylation site can be found at position 125 (serine). Also, a second acidic domain is found close to the C-terminus as an uninterrupted stretch of 14 glutamate residues. A similar C-terminal structure has been found by Kessel et al. Proc. Natl. Acad. Sci. USA 84: 5306-5310 (1987), in a murine homeodomain protein having nuclear localization.

In studies comparing the sequence of gene P1A to the sequences for P91A, 35B and P198, no similarities were found, showing that P1A is indicative of a different class of genes and antigens.

Example 10

With the P1A probe and sequence in hand, investigations were carried out to determine whether the gene present in normal tissue was identical to that expressed by the tumor. To do this, phage libraries were prepared, using lambda zapII 10 and genomic DNA of DBA2 murine kidney cells. P1A was used as a probe. Hybridization conditions were as described supra, and a hybridizing clone was found. The clone contained exons one and two of the P1A gene, and corresponded to positions -0.7 to 3.8 of figure 5. Following localization of this sequence, PCR amplification was carried out to obtain the sequence corresponding to 3.8 to 4.5 of figure 5.

Sequence analysis was carried out, and no differences were found between the gene from normal kidneys and the P1A gene as obtained from the P815 tumor cells.

In further experiments, the gene as found in DBA/2 kidney cells was transfected into PO.HTR, as described supra. These experiments, presented pictorially in figure 7, showed that antigens A and B were expressed as efficiently by the kidney gene isolated from normal kidney cells as with the P1A gene isolated from normal kidney cells.

These experiments lead to the conclusion that the gene coding for the tumor rejection antigen precursor is a gene that does not result from a mutation; rather, it would appear that the gene is the same as one present in normal cells, but is not expressed therein. The ramifications of this finding are important, and are discussed infra.

In studies not elaborated upon herein, it was found that variants of the gene were available. Some cells were "P1A^{-}B^{+}", rather than the normal "P1A". The only difference between these is a point mutation in exon 1, with the 18th triplet coding for Ala in the variant instead of Val.

Example 11

Additional experiments were carried out with other cell types. Following the protocols described for Northern blot hybridizations supra, RNA of normal liver and spleen cells was tested to determine if a transcript of the P1A gene could be found. The Northern blot data are presented in figure 4 and, as can be seen, there is no evidence of expression.

The murine P815 cell line from which P1A was isolated is a mastocytoma. Therefore, mast cell lines were studied to determine if they expressed the gene. Mast cell line MC/9, described by Nabel et al., Cell 23: 19-28 (1981), and short term cultures of bone marrow derived mast cells were tested in the manner described supra (Northern blotting), but no transcript was found. In contrast when a Balb/C derived IL-3 dependent cell line L138.8A (Hültner et al.,

J. Immunol. 142: 3440-3446 (1989)) was tested, a strong signal was found. The mast cell work is shown in figure 4.

It is known that both BALB/C and DBA/2 mice share H-2^d haplotype, and thus it was possible to test sensitivity to lysis using the CTLs described supra. Figure 8 shows these results, which essentially prove that anti-A and anti-B CTLs lysed the cells strongly, whereas anti-C and anti-D lines did not.

Further tests were carried out on other murine tumor cell lines, i.e., teratocarcinoma cell line PCC4 (Boon et al., Proc. Natl. Acad. Sci. USA 74: 272-275 (1977), and leukemias LEC and WEH1-3B. Expression could not be detected in any of these samples.

Example 12

The actual presentation of the P1A antigen by MHC molecules was of interest. To test this, cosmid C1A.3.1 was transfected into fibroblast cell line DAP, which shows phenotype H-2^k. The cell lines were transfected with genes expressing one of the K^d, D^d, and L^d antigen. Following transfection with both the cosmid and the MHC gene, lysis with CTLs was studied, again as described supra. These studies, summarized in Table 2, show that L^d is required for presentation of the P1A antigens A and B.

Table 2. H-2-restriction of antigens P815A and P815B

Recipient cell*	No of clones lysed by the CTL/ no. of H^+B^+ clones*	
	CTL anti-A	CTL anti-B
DAP ($H\text{-}2^k$)	0/208	0/194
DAP + K^d	0/165	0/162
DAP + D^d	0/157	0/129
DAP + L^d	25/33	15/20

*Cosmid C1A.3.1 containing the entire P1A gene was transfected in DAP cells previously transfected with $H\text{-}2^d$ class I genes as indicated.
*Independent drug-resistant colonies were tested for lysis by anti-A or anti-B CTL in a visual assay.

The observation that one may associate presentation of a tumor rejection antigen with a particular MHC molecule was confirmed in experiments with human cells and HLA molecules, as elaborated upon infra.

Example 13

Using the sequence of the P1A gene as well as the amino acid sequence derivable therefrom, antigenic peptides which were A^+ B^+ (i.e., characteristic of cells which express both the A and B antigens), and those which are A^- B^+ were identified. The peptide is presented in Figure 10. This peptide when administered to samples of PO.HTR cells

in the presence of CTL cell lines specific to cells presenting it, led to lysis of the PO.HTR cells, lending support to the view that peptides based on the product expressed by the gene can be used as vaccines.

Example 14

The human melanoma cell line referred to hereafter as MZ2-MEL is not a clonal cell line. It expresses four stable antigens recognized by autologous CTLs, known as antigens "D, E, F, and A". In addition, two other antigens "B" and "C" are expressed by some sublines of the tumor. CTL clones specific for these six antigens are described by Van den Eynde et al., Int. J. Canc. 44: 634-640 (1989). Among the recognized subclones of MZ2-MEL are MEL.43, MEL3.0 and MEL3.1. (Van den Eynde et al., supra). Cell line MEL3.1 expresses antigen E, as determined by CTL studies as described for P815 variants, supra, so it was chosen as a source for the nucleic acid sequence expressing the antigen precursor.

In isolating the pertinent nucleic acid sequence for a tumor rejection antigen precursor, the techniques developed supra, showed that a recipient cell is needed which fulfills two criteria: (i) the recipient cell must not express the TRAP of interest under normal conditions, and (ii) it must express the relevant class I HLA molecule. Also, the recipient cell must have a high transfection frequency, i.e., it must be a "good" recipient.

In order to secure such a cell line, the clonal subline ME3.1 was subjected to repeated selection with anti-E CTL 82/30 as described by Van den Eynde, supra. The repeated cycles of selection led to isolation of subclone MZ2-MEL-2.2 isc E^-. This subclone is also $HPRT^-$, (i.e., sensitive to HAT medium: 10^{-4} M hypoxanthine, 3.8 x 10^{-7} aminopterine, 1.6 x 10^{-5} M 2-deoxythymidine). The subclone is referred to as "MEL-2.2" for simplicity hereafter.

Example 15

The genomic DNA of MEL3.0 was prepared following Wölfel et al., Immunogenetics 26: 178-187 (1987), the disclosure of which is incorporated by reference. The plasmid pSVtkneoß, as described by Nicolas et al., Cold Spring Harb., Conf. Cell Prolif. 10: 469-485 (1983) confers geneticin resistance, so it can be used as a marker for cotransfection, as it was in this experiment.

Following a procedure similar but not identical to that of Corsao et al., Somatic Cell Molec. Genet 7: 603-616 (1981), total genomic DNA and the plasmid were cotransfected. The genomic DNA (60 μg) and plasmid DNA (6 μg) were mixed in 940 μl of 1 mM Tris·HCl (pH 7.5), 0.1 mM EDTA, after which 310 μl of 1M $CaCl_2$ was added. This solution was slowly added, under constant agitation, to 1.25 ml of 2xHBS (50 mM HEPES, 280 mM NaCl 1.5 mM Na_2HPO_4, adjusted to pH 7.1 with NaOH). The calcium phosphate DNA precipitates were allowed to form for 30-45 minutes at room

temperature, after which they were applied to 80 cm^2 tissue culture flasks which had been seeded 24 hours previously with $3x10^6$ MEL2.2 cells, in 22.5 ml of melanoma culture medium (Dulbecco's Modified Eagle's Medium) supplemented with 10% fetal calf serum. After 24 hours, the medium was replaced. Forty eight hours after transfection, the cells were harvested and seeded at $4x10^6$ cells per 80 cm^2 flask in melanoma culture medium supplemented with 2 mg/ml of geneticin. The geneticin serves as a selection marker.

Example 16

Thirteen days after transfection, geneticin-resistant colonies were counted, harvested, and cultured in nonselective medium for 2 or 3 days. Transfected cells were then plated in 96-well microplates at 200 cells/well in 200 ul of culture medium with 20% fetal calf serum (FCS) in order to obtain approximately 30 growing colonies per well. The number of microcultures was aimed at achieving redundancy, i.e., such that every independent transfectant should be represented at least four times.

After 10 days, wells contained approximately $6x10^4$ cells. These cells were detached, and 1/3 of each microculture was transferred to a duplicate plate. After 6 hours, i.e., after readherence, medium was removed and 1500 anti-E CTL (CTL 82/30), were added to each well in 100 μl of CTL culture medium with 35 U/ml of IL-2. One day later, the supernatant (50 μl) was harvested and examined

for TNF concentration, for reasons set forth in the following example.

Example 17

The size of the mammalian genome is $6x10^6$ kb. As the average amount of DNA integrated in each drug-resistant transfectant was expected to be about 200 kb, a minimum of 30,000 transfectants would need to be examined to ascertain whether antigen E had been transfected. Prior work with murine cells had shown that when a CTL stimulation assay was used, groups containing only 3% of cells expressing the antigen of interested could be identified. This should reduce the number of assays by a factor of 30. While an anti-E CTL assay, as described supra, in mixed E^+/E^- cells was helpful, it was not sufficient in that consistent results could not be obtained.

As a result, an alternative test was devised. Stimulation of CTLs was studied by release of tumor necrosis factor ("TNF") using well known methodologies which need not be repeated here. As described in Example 15, 1500 CTL 82/30 cells had been added per well of transfectants. These CTLs were collected 6 days after stimulation. As indicated supra, after 1/3 of the cells in each well had been removed and the remaining 2/3 ($4x10^4$) had readhered, the CTLs and IL-2 were added thereto. The 50 μl of supernatant was removed 24 hours later and transferred to a microplate containing $3x10^4$ W13 (WEHI-164 clone 13;

Espevik et al., J. Immunol. Meth. 95: 99-105 (1986)) cells in 50 μl of W13 culture medium (RPMI-1640, supplemented with L-arginine (116 mg/l), L-asparagine (36 mg/l), L-glutamine (216 mg/l), and 10% FCS supplemented with 2 μg of actinomycin D at 37% in an 8% CO_2 atmosphere. The cell line W13 is a mouse fibrosarcoma cell line sensitive to TNF. Dilutions of recombinant TNF-β in RPMI 1640 were added to target cell controls.

The W13 cultures were evaluated after 20 hours of incubation, and dead cell percentage was measured using an adaptation of the colorimetric assay of Hansen et al., J. Immunol. Meth. 119: 203-210 (1989). This involved adding 50 ml of (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide at 2.5 mg/ml in PBS, followed by two hours of incubation at 37°C. Dark blue formazan crystals were dissolved by adding 100 μl of lysis solution (1 volume N,N dimethyl formamide mixed at 37°C with two volumes of water containing 30% (w/v) sodium dodecyl sulphate, at pH 4.7 from 1.6% acetic acid and 2.5% 1N HCl). Plates were incubated at 37°C overnight, and ODs were taken at 570 nm using 650 nm as control. Dead cell percentage was determined via the formula:

$$100 \times \left[1 - \frac{100-(OD_{570} \text{ sample well})}{OD_{570} \text{ well + medium}} \right]$$

following Espevik et al., J. Immunol. Meth. 95: 99-105 (1986). The results showed that even when the ratio of E^+/E^- cells was as low as 1/45, significant production of TNF was observed, thus showing active CTLs. This led to the decision to test the drug resistant transfectants in groups of 30.

Example 18

Cells were tested for TNF production as discussed in Example 17, supra. A total of 100 groups of E^- cells ($4x10^6$ cells/group) were tested following transfection, and $7x10^4$ independent geneticin resistant transfectants were obtained, for an average of 700 per group. Only one group of transfected cells led to a microculture which caused anti-E antigen CTL clone 82/30 to produce TNF. Of 300 clones tested, 8 were positive. These clones were then tested for lysis by anti-E CTL, using the standard ^{51}Cr release assay, and were found to be lysed as efficiently as the original E^+ cell line. The transfectant E.T1, discussed herein, had the same lysis pattern as did MEL2.2 for CTLs against antigens B,C,D and F.

The fact that only one transfectant presented the antigen out of 70,000 geneticin resistance transfectants may at first seem very low, but it is not. The work described supra for P815 showed an average frequency of 1/13,000. Human DNA recipient MEL2.2 appears to integrate 5 times less DNA than P1.HTR.

Example 19

Once transfectant E.T1 was found, analysis had to address several questions including whether an E^+ contaminant of the cell population was the cause. The analysis of antigen presentation, described supra, shows that E.T1 is B^- and C^-, just like the recipient cell MEL2.2. It was also found to be $HPRT^-$, using standard selection procedures. All E^+ cells used in the work described herein, however, were $HPRT^+$.

It was also possible that an E^+ revertant of MEL2.2 was the source for E.T1. To test this, the observation by Perucho et al., Cell 22: 309-317 (1980), that cotransfected sequences usually integrate together at a single location of recipient genome was employed. If antigen E in a transfectant results from cotransfec-tion with pSVtkneoß, then sequences should be linked and deletion of the antigen might also delete the neighboring pSVtkneoß sequences. Wölfel et al., supra, has shown this to be true. If a normally E^- cell is transfected with pSVtkneoß, then sequences should be linked and deletion of the antigen might also delete the neighboring pSVtkneoß sequences. If a normally E^+ cell transfected with pSVtkneoß is E.T1, however, "co-deletion" should not take place. To test this, the transfectant E.T1 was subjected to immunoselection with 82/30, as described supra. Two antigen loss variants were obtained, which resisted lysis by this CTL. Neither of these had lost geneticin

resistance; however, Southern blot analysis showed loss of several neo^r sequences in the variants, showing close linkage between the E gene and neo^r gene in E.T1, leading to the conclusion that E.T1 was a transfectant.

Example 20

The E^+ subclone MZ2-MEL 4B was used as a source of DNA for preparation of a cosmid library. This library of nearly 700,000 cosmids was transfected into MZ2-MEL 2.2 cells, following the cosmid transfection protocols described supra.

By packaging the DNA of cosmid transfectants directly into lambda phase components, it is sometimes possible to retrieve cosmids that contain the sequences of interest. This procedure was unsuccessful here, so we rescued the transfected sequence by ligating DNA of the transfectant to appropriate restriction fragments of cosmid vector pTL6. This was tried with two transfectants and was successful with one of them. One cosmid, referred to as B3, was recovered from this experiment, and subjected to restriction endonuclease digestion via XmaI, or by BamHI digestion of a large, 12 kb XmaI transfected fragment. The fragments were cloned into vector pTZ 18R, and then transfected into MEL2.2. Again, TNF production was the measure by which successful transfection was determined. The experiments led to the determination of a gene sequence capable of transfecting antigen E on the 12 kb XmaI

fragment, and then on the 2.4 kb fragment of BamHI digestion of the 12 kb segment.

The 2.4 kb fragment hybridizes with a 2.4 kb fragment from MZ2-MEL and with a T cell clone of patient MZ-2, as determined by Southern Blots (BamHI/SmaI digested DNA). The band is absent from E^- antigen loss variants of MZ2-MEL, as seen in Figure 12.

The sequence for the E antigen precursor gene has been determined, and is presented herein:

```
      |  10      |  20      |  30      |  40      |  50      |  60
   1 GGATCCAGGC CCTGCCAGGA AAAATATAAG GGCCCTGCGT GAGAACAGAG GGGGTCATCC 60
  61 ACTGCATGAG AGTGGGGATG TCACAGAGTC CAGCCCACCC TCCTGGTAGC ACTGAGAAGC 120
 121 CAGGGCTGTG CTTGCCGTCT GCACCCTGAG GGCCCGTGGA TTCCTCTTCC TGGAGCTCCA 180
 181 GGAACCAGGC AGTGAGGCCT TGGTCTGAGA CAGTATCCTC AGGTCACAGA GCAGAGGATG 240
 241 CACAGGGTGT GCCAGCAGTG AATGTTTGCC CTGAATGCAC ACCAAGGGCC CCACCTGCCA 300
 301 CAGGACACAT AGGACTCCAC AGAGTCTGGC CTCACCTCCC TACTGTCAGT CCTGTAGAAT 360
 361 CGACCTCTGC TGGCCGGCTG TACCCTGAGT ACCCTCTCAC TTCCTCCTTC AGGTTTTCAG 420
 421 GGGACAGGCC AACCCAGAGG ACAGGATTCC CTGGAGGCCA CAGAGGAGCA CCAAGGAGAA 480
 481 GATCTGTAAG TAGGCCTTTG TTAGAGTCTC CAAGGTTCAG TTCTCAGCTG AGGCCTCTCA 540
 541 CACACTCCCT CTCTCCCCAG GCCTGTGGGT CTTCATTGCC CAGCTCCTGC CCACACTCCT 600
 601 GCCTGCTGCC CTGACGAGAG TCATCATGTC TCTTGAGCAG AGGAGTCTGC ACTGCAAGCC 660
 661 TGAGGAAGCC CTTGAGGCCC AACAAGAGGC CCTGGGCTGG TGTGTGTGCA GGCTGCCACC 720
 721 TCCTCCTCCT CTCCTCTGGT CCTGGGCACC CTGGAGGAGG TGCCCACTGC TGGGTCAACA 780
 781 GATCCTCCCC AGAGTCCTCA GGGAGCCTCC GCCTTTCCCA CTACCATCAA CTTCACTCGA 840
 841 CAGAGGCAAC CCAGTGAGGG TTCCAGCAGC CGTGAAGAGG AGGGGCCAAG CACCTCTTGT 900
 901 ATCCTGGAGT CCTTGTTCCG AGCAGTAATC ACTAAGAAGG TGGCTGATTT GGTTGGTTTT 960
 961 CTGCTCCTCA AATATCGAGC CAGGGAGCCA GTCACAAAGG CAGAAATGCT GGAGAGTGTC 1020
1021 ATCAAAAATT ACAAGCACTG TTTTCCTGAG ATCTTCGGCA AAGCCTCTGA GTCCTTGCAG 1080
1081 CTGGTCTTTG GCATTGACGT GAAGGAAGCA GACCCCACCG GCCACTCCTA TGTCCTTGTC 1140
1141 ACCTGCCTAG GTCTCTCCTA TGATGGCCTG CTGGGTGATA ATCAGATCAT GCCCAAGACA 1200
1201 GGCTTCCTGA TAATTGTCCT GGTCATGATT GCAATGGAGG GCGGCCATGC TCCTGAGGAG 1260
1261 GAAATCTGGG AGGAGCTGAG TGTGATGGAG GTGTATGATG GGAGGGAGCA CAGTGCCTAT 1320
1321 GGGGAGCCCA GGAAGCTGCT CACCCAAGAT TTGGTGCAGG AAAAGTACCT GGAGTACGGC 1380
1381 AGGTGCCGGA CAGTGATCCC GCACGCTATG AGTTCCTGTG GGGTCCAAGG GCCCTCGCTG 1440
1441 AAACCAGCTA TGTGAAAGTC CTTGAGTATG TGATCAAGGT CAGTGCAAGA GTTCGCTTTT 1500
1501 TCTTCCCATC CCTGCGTGAA GCAGCTTTGA GAGAGGAGGA AGAGGGAGTC TGAGCATGAG 1560
1561 TTGCAGCCAA GGCCAGTGGG AGGGGGACTG GGCCAGTGCA CCTTCCAGGG CCGCGTCCAG 1620
1621 CAGCTTCCCC TGCCTCGTGT GACATGAGGC CCATTCTTCA CTCTGAAGAG AGCGGTCAGT 1680
1681 GTTCTCAGTA GTAGGTTTCT GTTCTATTGC GTGACTTGGA GATTTATCTT TGTTCTCTTT 1740
1741 TGGAATTGTT CAAATGTTTT TTTTTAAGGG ATGTTTGAAT GAACTTCAGC ATCCAAGTTT 1800
1801 ATGAATGACA GCAGTCACAC AGTTCTGTGT ATATAGTTTA AGGGTAAGAG TCTTGTGTTT 1860
1861 TATTCAGATT GGGAAATCCA TTCTATTTTG TGAATTGGGA TAATAACAGC AGTGGAATAA 1920
1921 GTACTTAGAA ATGTGAAAAA TGAGCAGTAA AATAGATGAG ATAAAGAACT AAAGAAATTA 1980
1981 AGAGATAGTC AATTCTTGCC TTATACCTCA GTCTATTCTG TAAAATTTTT AAAGATATAT 2040
2041 GCATACCTGG ATTTCCTTGG CTTCTTTGAG AATGTAAGAG AAATTAAATC TGAATAAAGA 2100
2101 ATTCTTCCTG TTCACTGGCT CTTTTCTTCT CCATGCACTG AGCATCTGCT TTTTGGAAGG 2160
2161 CCCTGGGTTA GTAGTGGAGA TGCTAAGGTA AGCCAGACTC ATACCCACCC ATAGGGTCGT 2220
2221 AGAGTCTAGG AGCTGCAGTC ACGTAATCGA GGTGGCAAGA TGTCCTCTAA AGATGTAGGG 2280
2281 AAAAGTGAGA GAGGGGTGAG GGTGTGGGGC TCCGGGTGAG AGTGGTGGAG TGTCAATGCC 2340
2341 CTGAGCTGGG GCATTTTGGG CTTTGGGAAA CTGCAGTTCC TTCTGGGGGA GCTGATTGTA 2400
2401 ATGATCTTGG GTGGATCC                                            2418
      |  10      |  20      |  30      |  40      |  50      |  60
```

Example 21

After the 2.4 kb genomic segment had been identified, studies were carried out to determine if an "E^+" subline expressed any homologous DNA. Cell line MZ2-MEL 3.0 was used as a source, and a cDNA library was prepared from its mRNA, using art known techniques. The 2.4 kb segment was used as a probe, and mRNA of about 1.8 kb was identified as homologous, using Northern blot analysis. When cDNA was screened, clones were obtained showing almost complete identity to parts of the 2.4 kb fragment. Two exons were thus identified. An additional exon was located upstream of these, via sequencing segments of cosmid B3 located in front of the 2.4 kb BamHI fragment. The gene extends over about 4.5 kb, as shown in Figure 8. The starting point of the transcribed region was confirmed using PCR for the 5′ end of the cDNA. The three exons comprise 65, 73, and 1551 base pairs. An ATG is located at position 66 of exon 3, followed by an 828 base pair reading frame.

Example 22

To determine if smaller segments of the 2.4 kb fragment could transfer the expression of antigen E, smaller pieces corresponding to the larger gene were prepared, using art recognized techniques, and transferred into E^- cells. Figure 8 shows the boundaries of the three segments.

Transfer of antigen expression in this manner indicates that the gene codes for the antigen precursor, rather than coding for a protein which activates the antigen.

Example 23

The probing of cDNA described supra revealed, surprisingly, two different but closely related cDNAs. These cDNAs, when tested, did not transfer expression of antigen E, but they do show substantial homology to the first cDNA segment. The three segments, appear to indicate a newly recognized family of genes, referred to as "MAGE" for "melanoma antigen". In Figure 9, "mage -1" directs expression of the antigen from MZ2 cells. Portions of the third exon of each gene are presented in Figure 9. The second and third sequences are more closely related to each other than the first (18.1 and 18.9% difference compared to the first; 12% with each other). Out of 9 cDNA clones obtained, three of each type were obtained, suggesting equal expression. "MAGE" as used hereafter refers to a family of molecules, and the nucleic acids coding for them. These nucleic acids share a certain degree of homology and are expressed in tumor cells including several types of human tumor cells as well as in human tumors. The family is referred to as "MAGE" because the first members were identified in human melanoma cells. As the experiments which follow indicate, however, the members of the MAGE family are not at all restricted to melanoma tumors;

rather, MAGE refers to a family of tumor rejection antigen precursors and the nucleic acid sequences coding therefore. The antigens resulting therefrom are referred to herein as "MAGE TRAs" or "melanoma antigen tumor rejection antigens"

Example 24

Experiments with mouse tumors have demonstrated that new antigens recognized by T cells can result from point mutations that modify active genes in a region that codes for the new antigenic peptide. New antigens can also arise from the activation of genes that are not expressed in most normal cells. To clarify this issue for antigen MZ2-E, the mage-1 gene present in the melanoma cells was compared to that present in normal cells of patient MZ2.

Amplification by polymerase chain reaction (PCR) of DNA of phytohemagglutinin-activated blood lymphocytes using primers surrounding a 1300 bp stretch covering the first half of the 2.4 kb fragment was carried out. As expected, a PCR product was obtained whereas none was obtained with the DNA of the E^- variant. The sequence of this PCR product proved identical to the corresponding sequence of the gene carried by the E^+ melanoma cells. Moreover, it was found that antigen MZ2-E was expressed by cells transfected with the cloned PCR product. This result suggests that the activation of a gene normally silent is responsible for the appearance of tumor rejection antigen MZ2-E.

Example 25

In order to evaluate the expression of gene mage-1 by various normal and tumor cells, Northern blots were hybridized with a probe covering most of the third exon. In contrast with the result observed with human tumor cell line MZ2-MEL 3.0, no band was observed with RNA isolated from a CTL clone of patient MZ2 and phytohemagglutinin-activated blood lymphocytes of the same patient. Also negative were several normal tissues of other individuals (Figure 10 and Figure 11). Fourteen melanoma cell lines of other patients were tested. Eleven were positive with bands of varying intensities. In addition to these culture cell lines, four samples of melanoma tumor tissue were analyzed. Two samples, including a metastasis of patient MZ2 proved positive, excluding the possibility that expression of the gene represented a tissue culture artefact. A few tumors of other histological types, including lung tumors were tested. Most of these tumors were positive (Figures 10 and 11). These results indicated that the MAGE gene family is expressed by many melanomas and also by other tumors. However, they provided no clear indication as to which of genes mage-1, 2 or 3 were expressed by these cells, because the DNA probes corresponding to the three genes cross-hybridized to a considerable extent. To render this analysis more specific, PCR amplification and hybridization with highly specific oligo- nucleotide probes were used. cDNAs were obtained and amplified by PCR using oligonucleotide primers

corresponding to sequences of exon 3 that were identical for the three MAGE genes discussed herein. The PCR products were then tested for their ability to hybridize to three other oligonucleotides that showed complete specificity for one of the three genes (Figure 9). Control experiments carried out by diluting RNA of melanoma MZ2-MEL 3.0 in RNA from negative cells indicated that under the conditions used herein the intensity of the signal decreased proportionally to the dilution and that positive signals could still be detected at a dilution of 1/300. The normal cells (lymphocytes) that were tested by PCR were confirmed to be negative for the expression of the three MAGE genes, suggesting therefore a level of expression of less than $1/300^{th}$ that of the MZ2 melanoma cell line (Figure 11). For the panel of melanoma cell lines, the results clearly showed that some melanomas expressed MAGE genes mage 1, 2 and 3 whereas other expressed only mage-2 and 3 (Figures 11 and 10). Some of the other tumors also expressed all three genes whereas others expressed only mage-2 and 3 or only mage-3. It is impossible to exclude formally that some positive PCR results do not reflect the expression of one of the three characterized MAGE genes but that of yet another closely related gene that would share the sequence of the priming and hybridizing oligonucleotides. It can be concluded that the MAGE gene family is expressed by a large array of different tumors and that these genes are silent in the normal cells tested to this point.

Exammple 26

The availability of a sequence that transfects at high efficiency and efficiently expresses a TRAP made it possible to search for the associated major histocompatibility complex (MHC) class I molecule. The class I specificities of patient MZ2 are HLA-A1, A29, B37, B44 and C6. Four other melanomas of patients that had A1 in common with MZ2 were cotransfected with the 2.4 kb fragment and pSVtkneoß. Three of them yielded neo^r transfectants that stimulated TNF release by anti-E CTL clone 82/30, which is CD8+ (Figure 10). No E- transfectant was obtained with four other melanomas, some of which shared A29, B44 or C6 with MZ2. This suggests that the presenting molecule for antigen MZ2-E is HLA-A1. In confirmation, it was found that, out of 6 melanoma cell lines derived from tumors of HLA-A1 patients, two stimulated TNF release by anti-E CTL clone 82/30 of patient MZ2. One of these tumor cell lines, MI13443-MEL also showed high sensitivity to lysis by these anti-E CTL. These two melanomas were those that expressed mage-1 gene (Figure 13). Eight melanomas of patients with HLA haplotypes that did not include A1 were examined for their sensitivity to lysis and for their ability to stimulate TNF release by the CTL. None was found to be positive. The ability of some human anti-tumor CTL to lyse allogeneic tumors sharing an appropriate HLA specificity with the original tumor has been reported previously (Darrow, et al., J. Immunol. 142: 3329 (1989)). It is quite possible that antigenic peptides encoded by genes

mage 2 and 3 can also be presented to autologous CTL by HLA-A1 or other class I molecules, especially in view of the similar results found with murine tumors, as elaborated upon supra.

Example 27

As indicated supra, melanoma MZ2 expressed antigens F, D and A', in addition to antigen E. Following the isolation of the nucleic acid sequence coding for antigen E, similar experiments were carried out to isolate the nucleic acid sequence coding for antigen F.

To do this, cultures of cell line MZ2-MEL2.2, an E^- cell line described supra, were treated with anti-F CTL clone 76/6, in the same manner described for treatment with anti-E CTL clones. This resulted in the isolation of an F antigen loss variant, which was then subjected to several rounds of selection. The resulting cell line, "MZ2-MEL2.2.5" was completely resistant to lysis by anti-F CTLs, yet proved to be lysed by anti-D CTLs.

Again, following the protocols set forth for isolation of antigen -E precursor DNA, the F^- variant was transfected with genomic DNA from F^+ cell line MZ2-MEL3.0. The experiments yielded 90,000 drug resistant transfectants. These were tested for MZ2-F^- expression by using pools of 30 cells in the TNF detection assay elaborated upon supra. One pool stimulated TNF release by anti-F CTLs, and was cloned. Five of 145 clones were found to stimulate anti-

F CTLs. Lysis assays, also following protocols described supra, confirmed (i) expression of the gene coding for antigen F, and (ii) presentation of antigen F itself.

Example 28

Following identification of F^+ cell lines, the DNA therefrom was used to transfect cells. To do this, a cosmid library of F^+ cell line MZ2-MEL.43 was prepared, again using the protocols described supra. The library was divided into 14 groups of about 50,000 cosmids, and DNA from each group was transfected into MZ2-MEL2.2.5. Transfectants were then tested for their ability to stimulate TNF release from anti-F CTL clone 76/6. Of 14 groups of cosmids, one produced two independent transfectants expressing antigen F; a yield of two positives out of 17,500 geniticin resistant transfectants.

Example 29

The existence of a gene family was suggested by the pattern observed on the Southern blot (Figure 12). To do this, the 2.4 kb BamHI fragment, which transferred the expression of antigen M22-E, was labelled with 32p and used as a probe on a Southern Blot of BamHI digested DNA of E + cloned subclone M22-MEL2.2. Hybridization conditions included 50 $\mu l/cm^2$ of 3.5xSSC, 1xDenhardt's solution; 25 mM sodium phosphate buffer (pH 7.0), 0.5% SDS, 2mM EDTA, where the 2.4 kb probes had been labelled with [α^{32}p]dCTP (2-3000

Ci/mole), at $3x10^6$ cpm/ml. Hybridization was carried out for 18 hours at 65°C. After this, the membranes were washed at 65°C four times for one hour each in 2xSSC, 0.1% SDS, and finally for 30 minutes in 0.1xSSC, 0.1% SDS. To identify hybridization, membranes were autoradiographed using Kodak X-AR film and Kodak X-Omatic fine intensifying screens.

In the following examples, whenever "hybridization" is referred to, the stringency conditions used were similar to those described supra. "Stringent conditions" as used herein thus refers to the foregoing conditions; subject to routine, art recognized modification.

Example 30

The cDNA coding for mage 4 was identified from a sample of the human sarcoma cell line LB23-SAR. This cell line was found to not express mage 1, 2 or 3, but the mRNA of the cell line did hybridize to the 2.4 kb sequence for mage 1. To study this further, a cDNA library was prepared from total LB23-SAR mRNA, and was then hybridized to the 2.4 kb fragment. A cDNA sequence was identified as hybridizing to this probe, and is identified hereafter as mage 4.

Example 31

Experiments were carried out using PHA-activated lymphocytes from patient "MZ2", the source of the "MZ" cells discussed supra. An oligonucleotide probe which

showed homology to mage 1 but not mage 2 or 3 was hybridized with a cosmid library derived from the PHA activated cells. The size of the hybridizing BamHI cosmid fragment, however, was 4.5 kb, thus indicating that the material was not mage 1; however, on the basis of homology to mage 1-4, the fragment can be referred to as "mage 5". The sequence of MAGE 5 is presented in SEQ ID NO: 16.

Example 32

Melanoma cell line LB-33-MEL was tested. Total mRNA from the cell line was used to prepare cDNA, which was then amplified with oligos CHO9: (ACTCAGCTCCTCCCAGATTT), and CHO10: (GAAGAGGAGGGGCCAAG). These oligos correspond to regions of exon 3 that are common to previously described mage 1, 2 and 3.

To do this, 1 μg of RNA was diluted to a total volume of 20 μl, using 2 μl of 10x PCR buffer, 2 μl of each of 10 mM dNTP, 1.2 μl of 25 mM $MgCl_2$, 1 μl of an 80 mM solution of CHO9, described supra, 20 units of RNAsin, and 200 units of M-MLV reverse transcriptase. This was followed by incubation for 40 minutes at 42°C. PCR amplification followed, using 8 μl of 10x PCR buffer, 4.8 μl of 25 mM $MgCl_2$, 1 μl of CHO10, 2.5 units of Thermus acquaticus ("Taq") polymerase, and water to a total volume of 100 μl. Amplification was then carried out for 30 cycles (1 minute 94°C; 2 minutes at 52°C, 3 minutes at 72°C). Ten μl of each reaction were then size fractionated on agarose gel,

followed by nitrocellulose blotting. The product was found to hybridize with oligonucleotide probe CHO18 (TCTTGTATCCTGGAGTCC). This probe identified mage 1 but not mage 2 or 3. However, the product did not hybridize to probe SEQ 4 (TTGCCAAGATCTCAGGAA). This probe also binds mage 1 but not 2 and 3. This indicated that the PCR product contained a sequence that differed from mage 1, 2 and 3. Sequencing of this fragment also indicated differences with respect to mage 4 and 5. These results indicate a sequence differing from previously identified mage 1, 2, 3, 4 and 5, and is named mage 6.

Example 33

In additional experiments using cosmid libraries from PHA-activated lymphocytes of MZ2, the 2.4 kb mage 1 fragment was used as a probe and isolated a complementary fragment. This clone, however, did not bind to oligonucleotides specific for mage 1, 2, 3 or 4. The sequence obtained shows some homology to exon 3 of mage 1, and differs from mages 1-6. It is referred to as mage 7 hereafter. Additional screenings yielded mage 8-11.

Example 34

The usefulness of the TRAPs, as well as TRAs derived therefrom, was exemplified by the following.

Exon 3 of mage 1 was shown to transfer expression of antigen E. As a result, it was decided to test whether

synthetic peptides derived from this exon 3 could be used to confer sensitivity to anti-E CTL.

To do this, and using standard protocols, cells normally insensitive to anti-E/CTLs were incubated with the synthetic peptides derived from Exon 3.1. Using the CTL lytic assays described supra on P815A, and a peptide concentration of 3 mM, the peptide Glu-Ala-Asp-Pro-Thr-Gly-His-Ser-Tyr was shown to be best. The assay showed lysis of 30%, indicating conferring of sensitivity to the anti-E CTL.

Example 35

Nucleic acid sequences referred to as "smage" were isolated from murine cells. Using the protocols described supra, a cosmid library was prepared from the DNA of normal DBA/2 kidney cells, using cosmid vector C2RB. As a probe, the 2.4 kb BamHI fragment of MAGE-1 was used. The DNA was blotted to nylon filters, and these were washed in 2xSSC at 65°C to identify the smage material.

Example 36

Further tissue samples were tested for the presence of MAGE genes, using the protocols discussed supra. Some of these results follow.

There was no expression of the MAGE genes in brain or kidney tumor tissue. Colon tumor tissue showed expression of MAGE 1, 2, 3 and 4, although not all tumors tested showed expression of all MAGE genes. This is also true for

pancreatic tumor (MAGE 1); non-small cell lung (MAGE 1, 2, 3 and 4), prostate (MAGE 1), sarcomas (MAGE 1, 2, 3 and 4), breast (MAGE 1, 2 and 3), and larynx (MAGE 1 and 4).

The foregoing disclosure, including the examples, places many tools of extreme value in the hands of the skilled artisan. To begin, the examples identify and provide a methodology for isolating nucleic acid molecules which code for tumor rejection antigen precursors as well as the nucleic acid molecules complementary thereto. It is known that DNA exists in double stranded form, and that each of the two strands is complementary to the other. Nucleic acid hybridization technology has developed to the point where, given a strand of DNA, the skilled artisan can isolate its complement, or synthesize it.

"Nucleic acid molecule" as used herein refers to all species of DNA and RNA which possess the properties discussed supra. Genomic and complementary DNA, or "cDNA" both code for particular proteins, and as the examples directed to isolation of MAGE coding sequences show, this disclosure teaches the artisan how to secure both of these.

Similarly, RNA molecules, such as mRNA can be secured. Again, with reference to the skilled artisan, once one has a coding sequence in hand, mRNA can be isolated or synthesized.

Complementary sequences which do not code for TRAP, such as "antisense DNA" or mRNA are useful, e.g., in

probing for the coding sequence as well as in methodologies for blocking its expression.

It will also be clear that the examples show the manufacture of biologically pure cultures of cell lines which have been transfected with nucleic acid sequences which code for or express the TRAP molecules. Such cultures can be used as a source for tumor rejection antigens, e.g., or as therapeutics. This aspect of the invention is discussed infra.

Cells transfected with the TRAP coding sequences may also be transfected with other coding sequences. Examples of other coding sequences include cytokine genes, such as interleukins (e.g., IL-2 or IL-4), or major histocompatibility complex (MHC) or human leukocyte antigen (HLA) molecules. Cytokine gene transfection is of value because expression of these is expected to enhance the therapeutic efficacy of the biologically pure culture of the cells in vivo. The art is well aware of therapies where interleukin transfectants have been administered to subjects for treating cancerous conditions. In a particularly preferred embodiment, cells are transfected with sequences coding for each of (i) a TRAP molecule, (ii) an HLA/MHC molecule, and (iii) a cytokine.

Transfection with an MHC/HLA coding sequence is desirable because certain of the TRAs may be preferentially or specifically presented only by particular MHC/HLA molecules. Thus, where a recipient cell already expresses the MHC/HLA molecule associated with presentation of a TRA,

additional transfection may not be necessary although further transformation could be used to cause over-expression of the antigen. On the other hand, it may be desirable to transfect with a second sequence when the recipient cell does not normally express the relevant MHC/HLA molecule. It is to be understood, of course, that transfection with one additional sequence does not preclude further transfection with other sequences.

The term "biologically pure" as used in connection with the cell line described herein simply means that these are essentially free of other cells. Strictly speaking, a "cell line" by definition is "biologically pure", but the recitation will establish this fully.

Transfection of cells requires that an appropriate vector be used. Thus, the invention encompasses expression vectors where a coding sequence for the TRAP of interest is operably linked to a promoter. The promoter may be a strong promoter, such as those well known to the art, or a differential promoter, i.e., one which is operative only in specific cell types. The expression vectors may also contain all or a part of a viral or bacterial genome, such as vaccinia virus or BCG. Such vectors are especially useful in preparing vaccines.

The expression vectors may incorporate several coding sequences, as long as the TRAP sequence is contained therein. The cytokine and/or MHC/HLA genes discussed supra may be included in a single vector with the TRAP sequence. Where this is not desired, then an expression system may be

provided, where two or more separate vectors are used where each coding sequence is operably linked to a promoter. Again, the promoter may be a strong or differential promoter. Co-transfection is a well known technique, and the artisan in this field is expected to have this technology available for utilization. The vectors may be constructed so that they code for the TRA molecule directly, rather than the TRAP molecule. This eliminates the need for post-translational processing.

As the foregoing discussion makes clear, the sequences code for "tumor rejection antigen precursors" ("TRAPs") which, in turn, are processed into tumor rejection antigens ("TRAs"). Isolated forms of both of these categories are described herein, including specific examples of each. Perhaps their most noteworthy aspect is as vaccines for treating various cancerous conditions. The evidence points to presentation of TRAs on tumor cells, followed by the development of an immune response and deletion of the cells. The examples show that when various TRAs are administered to cells, a CTL response is mounted and presenting cells are deleted. This is behavior characteristic of vaccines, and hence TRAPs, which are processed into TRAs, and the TRAs themselves may be used, either alone or in pharmaceutically appropriate compositions, as vaccines. Similarly, presenting cells may be used in the same manner, either alone or as combined with ingredients to yield pharmaceutical compositions. Additional materials which may be used as vaccines include

isolated cells which present the TRA molecule on their surface, as well as TRAP fragments, mutated viruses, especially etiolated forms, and transfected bacteria. "Fragments" as used herein refers to peptides which are smaller than the TRA, but which possess the properties required of a vaccine, as discussed supra. Another vaccine comprises or consists of complexes of TRA and HLA molecule. Vaccines of the type described herein may be used preventively, i.e., via administration to a subject in an amount sufficient to prevent onset of a cancerous condition.

The generation of an immune response, be it T-cell or B-cell related, is characteristic of the effect of the presented tumor rejection antigen. With respect to the B-cell response, this involves, inter alia, the generation of antibodies to the TRA, i.e., which specifically bind thereto. In addition, the TRAP molecules are of sufficient size to render them immunogenic, and antibodies which specifically bind thereto are a part of this invention. These antibodies may be polyclonal or monoclonal, the latter being prepared by any of the well recognized methodologies for their preparation which need not be repeated here. For example, mAbs may be prepared using an animal model, e.g., a Balb/C mouse or in a test tube, using, e.g., EBV transformants. In addition, antiserum may be isolated from a subject afflicted with a cancerous condition where certain cells present a TRA. Such

antibodies may also be generated to epitopes defined by the interaction of TRA and HLA/MHC molecules.

Review of the foregoing disclosure will show that there are a number of facets to the system which may be referred to as "tumor rejection antigen presentation and recognition". Recognition of these phenomena has diagnostic consequences. For example, the existence of specific CTL clones, or antibodies to the TRA makes it possible to diagnose or monitor cancerous conditions (explained infra), by monitoring the CTLs in a sample from a subject, binding of antibodies to TRAs, or the activity of anti-TRA CTLs in connection with subject samples. Similarly, the expression of nucleic acid molecules for TRAPs can be monitored via amplification (e.g., "polymerase chain reaction"), anti-sense hybridization, probe technologies, and so forth. Various subject samples, including body fluids (blood, serum, and other exudates, e.g.), tissues and tumors may be so assayed.

A particular manner of diagnosis is to use an adaptation of the standard "tuberculin test" currently used for diagnosis of tuberculosis. This standard skin test administers a stable form of "purified protein derivative" or "PPD" as a diagnostic aid. In a parallel fashion, TRAs in accordance with this invention may be used in such a skin test as a diagnostic aid or monitoring method.

The term "cancerous condition" is used herein to embrace all physiological events that commence with the initiation of the cancer and result in final clinical

manifestation. Tumors do not spring up "ab initio" as visible tumors; rather there are various events associated with the transformation of a normal cell to malignancy, followed by development of a growth of biomass, such as a tumor, metastasis, etc. In addition, remission may be conceived of as part of "a cancerous condition" as tumors seldom spontaneously disappear. The diagnostic aspects of this invention include all events involved in carcinogenesis, from the first transformation to malignancy of a single cell, through tumor development and metastasis, as well as remission. All are embraced herein.

Where "subject" is used, the term embraces any species which can be afflicted with a cancerous condition. This includes humans and non-humans, such as domesticated animals, breeding stock, and so forth.

There are therapeutic aspects of this invention as well. The efficacy of administration of effective amounts of TRAPs and TRAs as vaccines has already been discussed supra. Similarly, one may develop the specific CTLs in vitro and then administer these to the subject. Antibodies may be administered, either polyclonal or monoclonal, which specifically bind to cells presenting the TRA of interest. These antibodies may be coupled to specific antitumor agents, including, but not being limited to, methotrexate radio-iodinated compounds, toxins such as ricin, other cytostatic or cytolytic drugs, and so forth. Thus, "targeted" antibody therapy is included herein, as is the

application of deletion of the cancerous cells by the use of CTLs.

The terms and expressions which have been employed are used as terms of description and not of limitation, and there is no intention in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, it being recognized that various modifications are possible within the scope of the invention.

Claims:

1. Isolated nucleic acid molecule which codes for a tumor rejection antigen precursor or is complementary to a nucleic acid molecule which codes for a tumor rejection antigen precursor.

2. The isolated nucleic acid molecule of claim 1, wherein said molecule codes for a tumor rejection antigen precursor.

3. Isolated nucleic acid molecule of claim 1, wherein said molecule codes for a human tumor rejection antigen precursor.

4. The isolated nucleic acid molecule of claim 1, wherein said molecule is complementary to a nucleic acid molecule which codes for tumor rejection antigen precursor.

5. The isolated nucleic acid molecule of claim 1, wherein said molecule is DNA.

6. The isolated nucleic acid molecule of claim 1, wherein said molecule is RNA.

7. The isolated nucleic acid molecule of claim 1, wherein said molecule is a gene.

8. The isolated nucleic acid molecule of claim 5, wherein said DNA is genomic DNA.

9. The isolated nucleic acid molecule of claim 5, wherein said DNA is cDNA.

10. The isolated nucleic acid molecule of claim 6, wherein said RNA is mRNA.

11. The isolated nucleic acid molecule of claim 4, wherein said molecule hybridizes to isolated nucleic acid which codes for tumor rejection antigen precursor under stringent conditions.

12. The isolated nucleic acid molecule of claim 1, wherein said molecule codes for a MAGE antigen precursor or is complementary to a molecule which codes for a MAGE antigen precursor.

13. The isolated nucleic acid molecule of claim 12, wherein said MAGE antigen precursor is selected from the group consisting of mage 1, mage 2, mage 3, mage 4, mage 5, mage 6, mage 7, mage 8, mage 9, mage 10, mage 11, smage I and smage II.

14. The isolated nucleic acid molecule of claim 12, wherein said molecule codes for a MAGE antigen precursor.

15. The isolated nucleic acid molecule of claim 12, wherein said molecule is complementary to a molecule which codes for a MAGE antigen precursor.

16. The isolated nucleic acid molecule of claim 12, wherein said molecule is DNA.

17. The isolated nucleic acid molecule of claim 12, wherein said molecule is RNA.

18. The isolated nucleic acid molecule of claim 12, wherein said molecule is a gene.

19. The isolated nucleic acid molecule of claim 16, wherein said DNA is genomic DNA.

20. The isolated nucleic acid molecule of claim 16, wherein said DNA is cDNA.

21. The isolated nucleic acid molecule of claim 17, wherein said RNA is mRNA.

22. The isolated nucleic acid molecule of claim 12, comprising a nucleotide sequence set forth in figure 9.

23. The isolated nucleic acid molecule of claim 15, wherein said molecule hybridizes to a molecule which codes for a MAGE antigen precursor under stringent conditions.

24. Isolated nucleic acid molecule of claim 1, coding for a tumor rejection antigen precursor for mastocytoma.

25. Isolated nucleic acid molecule of claim 1, coding for tumor rejection antigen precursor P1A.

26. Isolated nucleic acid molecule of claim 1, having the nucleotide sequence of figure 5.

27. Biologically pure culture of a cell line transfected with the nucleic acid sequence of claim 2.

28. Biologically pure culture of a cell line transfected with the nucleic acid sequence of claim 12.

29. Biologically pure culture of a cell line transfected with the nucleic acid sequence of claim 22.

30. Biologically pure culture of a cell line of claim 27, selected from the group consisting of P1A.T2 and P1A.TC3.1.

31. Biologically pure culture of a highly transfectable cell line derived from a parent cell line which expresses at least one P815 tumor antigen, wherein said highly transfectable cell line does not express any of P815 tumor antigens A, B and C.

32. Biologically pure cell line of claim 31, comprising cell line PO.HTR.

33. Biologically pure culture of a cell line of claim 27, wherein said tumor rejection antigen precursor is a human tumor antigen precursor.

34. Biologically pure culture of a cell line of claim 33, wherein said human tumor antigen precursor is found in melanoma cells.

35. Biologically pure cell line of claim 34, said tumor rejection antigen precursor is mage-1 and said isolated DNA has nucleic acid sequence:

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      |  10      |  20      |  30      |  40      |  50      |  60
    1 GGATCCAGGC CCTGCCAGGA AAAATATAAG GGCCCTGCGT GAGAACAGAG GGGGTCATCC 60
   61 ACTGCATGAG AGTGGGGATG TCACAGAGTC CAGCCCACCC TCCTGGTAGC ACTGAGAAGC 120
  121 CAGGGCTGTG CTTGCGGTCT GCACCCTGAG GGCCCGTGGA TTCCTCTTCC TGGAGCTCCA 180
  181 GGAACCAGGC AGTGAGGCCT TGGTCTGAGA CAGTATCCTC AGGTCACAGA GCAGAGGATG 240
  241 CACAGGGTGT GCCAGCAGTG AATGTTTGCC CTGAATGCAC ACCAAGGGCC CCACCTGCCA 300
  301 CAGGACACAT AGGACTCCAC AGAGTCTGGC CTCACCTCCC TACTGTCAGT CCTGTAGAAT 360
  361 CGACCTCTGC TGGCCGGCTG TACCCTGAGT ACCCTCTCAC TTCCTCCTTC AGGTTTTCAG 420
  421 GGGACAGGCC AACCCAGAGG ACAGGATTCC CTGGAGGCCA CAGAGGAGCA CCAAGGAGAA 480
  481 GATCTGTAAG TAGGCCTTTG TTAGAGTCTC CAAGGTTCAG TTCTCAGCTG AGGCCTCTCA 540
  541 CACACTCCCT CTCTCCCCAG GCCTGTGGGT CTTCATTGCC CAGCTCCTGC CCACACTCCT 600
  601 GCCTGCTGCC CTGACGAGAG TCATCATGTC TCTTGAGCAG AGGAGTCTGC ACTGCAAGCC 660
  661 TGAGGAAGCC CTTGAGGCCC AACAAGAGGC CCTGGGCTGG TGTGTGTGCA GGCTGCCACC 720
  721 TCCTCCTCCT CTCCTCTGGT CCTGGGCACC CTGGAGGAGG TGCCCACTGC TGGGTCAACA 780
  781 GATCCTCCCC AGAGTCCTCA GGGAGCCTCC GCCTTTCCCA CTACCATCAA CTTCACTCGA 840
  841 CAGAGGCAAC CCAGTGAGGG TTCCAGCAGC CGTGAAGAGG AGGGGCCAAG CACCTCTTGT 900
  901 ATCCTGGAGT CCTTGTTCCG AGCAGTAATC ACTAAGAAGG TGGCTGATTT GGTTGGTTTT 960
  961 CTGCTCCTCA AATATCGAGC CAGGGAGCCA GTCACAAAGC CAGAAATGCT GGAGAGTGTC 1020
 1021 ATCAAAAATT ACAAGCACTG TTTTCCTGAG ATCTTCGGCA AAGCCTCTGA GTCCTTGCAG 1080
 1081 CTGGTCTTTG GCATTGACGT GAAGGAAGCA GACCCCACCG GCCACTCCTA TGTCCTTGTC 1140
 1141 ACCTGCCTAG GTCTCTCCTA TGATGGCCTG CTGGGTGATA ATCAGATCAT GCCCAAGACA 1200
 1201 GGCTTCCTGA TAATTGTCCT GGTCATGATT GCAATGGAGG GCGGCCATGC TCCTGAGGAG 1260
 1261 GAAATCTGGG AGGAGCTGAG TGTGATGGAG GTGTATGATG GGAGGGAGCA CAGTGCCTAT 1320
 1321 GGGGAGCCCA GGAAGCTGCT CACCCAAGAT TTGGTGCAGG AAAAGTACCT GGAGTACCGC 1380
 1381 AGGTGCCGGA CAGTGATCCC GCACGCTATG AGTTCCTGTG GGGTCCAAGG GCCCTCGCTG 1440
 1441 AAACCAGCTA TGTGAAAGTC CTTGAGTATG TGATCAAGGT CAGTGCAAGA GTTCGCTTTT 1500
 1501 TCTTCCCATC CCTGCGTGAA GCAGCTTTGA GAGAGGAGGA AGAGGGAGTC TGAGCATGAG 1560
 1561 TTGCAGCCAA GGCCAGTGGG AGGGGGACTG GGCCAGTGCA CCTTCCAGGG CCGCGTCCAG 1620
 1621 CAGCTTCCCC TGCCTCGTGT GACATGAGGC CCATTCTTCA CTCTGAAGAG AGCGGTCAGT 1680
 1681 GTTCTCAGTA GTAGGTTTCT GTTCTATTGG GTGACTTGGA GATTTATCTT TGTTCTCTTT 1740
 1741 TGGAATTGTT CAAATGTTTT TTTTTAAGGG ATGGTTGAAT GAACTTCAGC ATCCAAGTTT 1800
 1801 ATGAATGACA GCAGTCACAC AGTTCTGTGT ATATAGTTTA AGGGTAAGAG TCTTGTGTTT 1860
 1861 TATTCAGATT GGGAAATCCA TTCTATTTTG TGAATTGGGA TAATAACAGC AGTGGAATAA 1920
 1921 GTACTTAGAA ATGTGAAAAA TGAGCAGTAA AATAGATGAG ATAAAGAACT AAAGAAATTA 1980
 1981 AGAGATAGTC AATTCTTGCC TTATACCTCA GTCTATTCTG TAAAATTTTT AAAGATATAT 2040
 2041 GCATACCTGG ATTTCCTTGG CTTCTTTGAG AATGTAAGAG AAATTAAATC TGAATAAAGA 2100
 2101 ATTCTTCCTG TTCACTGGCT CTTTTCTTCT CCATGCACTG AGCATCTGCT TTTTGGAAGG 2160
 2161 CCCTGGGTTA GTAGTGGAGA TGCTAAGGTA AGCCAGACTC ATACCCACCC ATAGGGTCGT 2220
 2221 AGAGTCTAGG AGCTGCAGTC ACGTAATCGA GGTGGCAAGA TGTCCTCTAA AGATGTAGGG 2280
 2281 AAAAGTGAGA GAGGGGTGAG GGTGTGGGGC TCCGGGTGAG AGTGGTGGAG TGTCAATGCC 2340
 2341 CTGAGCTGGG GCATTTTGGG CTTTGGGAAA CTGCAGTTCC TTCTGGGGGA GCTGATTGTA 2400
 2401 ATGATCTTGG GTGGATCC                                            2418
      |  10      |  20      |  30      |  40      |  50      |  60
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36. The biologically pure culture of claim 27, wherein said cell line is transfected by a nucleic acid sequence coding for a cytokine.

37. The biologically pure culture of claim 36, wherein said cell line is further transfected by a nucleic acid sequence coding for an HLA molecule.

38. The biologically pure culture of claim 36, wherein said cytokine is an interleukin.

39. The biologically pure culture of claim 38, wherein said interleukin is IL-2.

40. The biologically pure culture of claim 38, wherein said interleukin is IL-4.

41. The biologically pure culture of claim 27, wherein said cell line is transfected by a nucleic acid sequence which codes for an MHC molecule or an HLA molecule.

42. The biologically pure culture of claim 27, wherein said cell line expresses an MHC or HLA molecule which presents a tumor rejection antigen derived from a tumor rejection antigen precursor (TRAP), wherein said TRAP is coded for by a nucleic acid sequence transfected into said cell line.

43. The biologically pure culture of claim 27, wherein said culture is non-proliferative.

44. The biologically pure culture of claim 27, wherein said cell line is a fibroblast cell line.

45. Transfected bacteria containing the nucleic acid sequence of claim 2.

46. Mutated virus containing the nucleic acid sequence of claim 2.

47. Expression vector useful in transfecting a cell comprising the isolated nucleic acid molecule of claim 2 operably linked to a promoter.

48. Expression vector useful in transfecting a cell comprising a nucleic acid sequence coding for a tumor rejection antigen operably linked to a promoter.

49. Expression vector of claim 47, wherein said promoter is a strong promoter.

50. Expression vector of claim 47, wherein said promoter is a differential promoter.

51. Expression vector useful in transfecting a cell comprising the isolated nucleic acid molecule of claim 7 operably linked to a promoter.

52. Expression vector useful in transfecting a cell comprising the isolated nucleic acid molecule of claim 13 operably linked to a promoter.

53. Expression vector useful in transfecting a cell comprising the isolated nucleic acid molecule of claim 14 operably linked to a promoter.

54. Expression vector useful in transfecting a cell comprising the isolated nucleic acid molecule of claim 18 operably linked to a promoter.

55. Expression vector useful in transfecting a cell comprising the isolated nucleic acid molecule of claim 22 operably linked to a promoter.

56. The expression vector of claim 47, further comprising a nucleic acid molecule which codes for an MHC or HLA.

57. The expression vector of claim 47, further comprising a nucleic acid molecule which codes for a cytokine.

58. The expression vector of claim 57, wherein said cytokine is an interleukin.

59. The expression vector of claim 58, wherein said interleukin is IL-2.

60. The expression vector of claim 58, wherein said interleukin is IL-4.

61. The expression vector of claim 47, further comprising a bacterial or viral genome or portion thereof.

62. The expression vector of claim 61, wherein said viral genome vaccinia virus DNA and said bacterial genome or portion thereof in BCG DNA.

63. Expression system useful in transfecting a cell, comprising (i) a first vector containing a nucleic acid molecule which codes for a tumor rejection antigen precursor, and (ii) a second vector selected from the group consisting of (a) a vector containing a nucleic acid molecule which codes for an MHC or HLA molecule which presents a tumor rejection antigen derived from said tumor rejection antigen precursor, and (b) a vector containing a nucleic acid sequence which codes for an interleukin.

64. Isolated tumor rejection antigen precursor.

65. Isolated human tumor rejection antigen precursor.

66. Isolated tumor rejection antigen precursor of claim 65, wherein said precursor is mage-1.

67. Isolated tumor rejection antigen precursor of claim 65, wherein said precursor is a precursor for antigen F.

68. Isolated tumor rejection antigen precursor coded for by the nucleic acid molecule of claim 2.

69. Isolated tumor rejection antigen precursor coded for by the nucleic acid molecule of claim 12.

70. Isolated tumor rejection antigen precursor coded for by the nucleic acid molecule of claim 13.

71. Isolated tumor rejection antigen precursor coded for by the nucleic acid molecule of claim 22.

72. Isolated tumor rejection antigen.

73. Isolated human tumor rejection antigen.

74. Isolated tumor rejection antigen of claim 72 having amino acid sequence of SEQ ID NO: 4.

75. Isolated tumor rejection antigen of claim 72, wherein said tumor rejection antigen is antigen E.

76. Isolated tumor rejection antigen of claim 72, wherein said tumor rejection antigen is antigen F.

77. Vaccine useful in treating a subject afflicted with a cancerous condition comprising a tumor rejection antigen precursor which provokes an immune response when administered to a subject.

78. Vaccine useful in treating a subject afflicted with a cancerous condition comprising a peptide fragment derived from a tumor rejection antigen precursor, wherein said fragment is larger than the tumor rejection antigen derived from said tumor rejection antigen precursor and smaller than said tumor rejection antigen precursor and which provokes an immune response when administered to a subject.

79. Vaccine of claim 77, wherein said TRAP is a human TRAP.

80. Vaccine of claim 77 wherein said precursor is mage-1.

81. Vaccine of claim 79, wherein said precursor is antigen F precursor.

82. Vaccine useful in treating a patient with a cancer comprising a tumor rejection antigen of claim 72 which provokes an immune response when administered to a subject.

83. Vaccine of claim 82, wherein said tumor rejection antigen has amino acid sequence of SEQ ID NO: 4.

84. The vaccine of claim 81, wherein said tumor rejection antigen is antigen E.

85. The vaccine of claim 81, wherein said tumor rejection antigen is antigen F.

86. The vaccine of claim 77, wherein said tumor rejection antigen precursor is the expression product of an expression vector containing a viral genome or portion thereof.

87. Vaccine useful in treating a patient with a cancer comprising the transfected bacterial of claim 45 and a pharmaceutically acceptable adjuvant.

88. Vaccine useful in treating a cancerous condition comprising the mutated virus of claim 46, and a pharmacologically acceptable adjuvant.

89. Vaccine useful in treating a subject afflicted with a cancerous condition comprising a complex of a tumor rejection antigen and an HLA molecule.

90. Isolated peptide useful in treating a subject afflicted with a cancerous condition, said peptide having the amino acid of SEQ ID NO: 26.

91. Vaccine useful in treating a subject afflicted with a cancerous condition comprising the isolated cell line of claim 27 and a pharmacologically acceptable adjuvant.

92. Vaccine useful in treating a subject afflicted with a cancerous condition comprising the isolated cell line of claim 37 and a pharmacologically acceptable adjuvant.

93. Composition of matter useful in treating a cancerous condition comprising a non proliferative cell line having expressed on its surface a tumor rejection antigen precursor specific for a tumor characteristic of said cancerous condition, and a pharmaceutically acceptable carrier.

94. Composition of matter of claim 93, wherein said cell line is a human cell line.

95. Composition of matter of claim 93, wherein said pharmaceutically acceptable carrier is a liposome.

96. Composition of matter useful in treating a cancerous condition comprising a non proliferative cell line having expressed on its surface a tumor rejection antigen specific for a tumor characteristic of said cancerous condition, and a pharma- ceutically acceptable carrier.

97. Composition of matter of claim 96, wherein said cell line is a human cell line.

98. Composition of matter of claim 96, wherein said pharma ceutically acceptable carrier is a liposome.

99. Composition of matter useful in treating a cancerous condition, comprising (i) a tumor rejection antigen or tumor rejection antigen precursor, (ii) an MHC or HLA molecule, and (iii) a pharmaceutically acceptable carrier.

100. Composition of matter of claim 99, wherein said pharmaceutically acceptable carrier is a liposome.

101. Antibody which specifically binds to a tumor rejection antigen precursor.

102. Antibody of claim 101, wherein said antibody is a monoclonal antibody.

103. Antibody of claim 101, wherein said tumor rejection antigen precursor is mage-1.

104. Antibody of claim 103, wherein said antibody is a monoclonal antibody.

105. Antibody of claim 101, wherein said tumor rejection antigen precursor is antigen F precursor.

106. Antibody of claim 105, wherein said antibody is a monoclonal antibody.

107. Antibody of claim 101, wherein said tumor rejection antigen precursor is a MAGE precursor.

108. Antibody of claim 107, wherein said antibody is a monoclonal antibody.

109. Antibody of claim 107, wherein said MAGE precursor is mage 1, mage 2, mage 3, mage 4, mage 5, mage 6, mage 7, mage 8, mage 9, mage 10, mage 11, smage I and smage II.

110. Antibody of claim 109, wherein said antibody is a monoclonal antibody.

111. Antibody which specifically binds to a tumor rejection antigen.

112. Antibody of claim 111, wherein said antibody is a monoclonal antibody.

113. Antibody of claim 111, wherein said tumor rejection antigen is that set forth in SEQ ID NO: 4.

114. Antibody of claim 113, wherein said antibody is a monoclonal antibody.

115. Antibody of claim 111, wherein said tumor rejection antigen is antigen E.

116. Antibody of claim 115, wherein said antibody is a monoclonal antibody.

117. Antibody of claim 111, wherein said tumor rejection antigen is antigen F.

118. Antibody of claim 117, wherein said antibody is a monoclonal antibody.

119. Antibody which specifically binds to a complex of (i) tumor rejection antigen and (ii) HLA molecule, but does not bind to (i) or (ii) alone.

120. The antibody of claim 119, wherein said antibody is a monoclonal antibody.

121. Method for diagnosing a cancerous condition in a subject, comprising contacting a lymphocyte containing sample of said subject to a cell line transfected with a DNA sequence coding for a tumor rejection antigen precursor expressed by cells associated with said cancerous condition, and determining lysis of said transfected cell line by a cytotoxic T cell line specific for a tumor rejection antigen derived from said tumor rejection antigen precursor, said lysis being indicative of said cancerous condition.

122. Method of claim 121, wherein said tumor rejection antigen precursor is a MAGE antigen.

123. Method for determining regression, progression or onset of a cancerous condition comprising monitoring a sample from a patient with said cancerous condition for a parameter selected from the group consisting of (i) tumor rejection antigen precursor, (ii) tumor rejection antigen and (iii) cytolytic T cells specific for a tumor rejection antigen associated with said cancerous condition, wherein amount of said parameter is indicative of progression or regression or onset of said cancerous condition.

124. Method of claim 123, wherein said sample is a body fluid.

125. Method of claim 123, wherein said sample is a tissue.

126. Method of claim 123, comprising contacting said sample with an antibody which specifically binds with said tumor rejection antigen or tumor rejection antigen precursor.

127. Method of claim 126, wherein said antibody is labelled with a radioactive label or an enzyme.

128. Method of claim 126, wherein said antibody is a monoclonal antibody.

129. Method of claim 123, comprising amplifying RNA which codes for said tumor rejection antigen precursor.

130. Method of claim 129, wherein said amplifying comprises carrying out polymerase chain reaction.

131. Method of claim 123, comprising contacting said sample with a nucleic acid molecule which specifically hybridizes to a nucleic acid molecule which codes for or expresses said tumor rejection antigen precursor.

132. Method of claim 123, comprising assaying said sample for shed tumor rejection antigen.

133. Method for diagnosing a cancerous condition comprising assaying a sample taken from a subject for a cytolytic T cell specific for a tumor rejection antigen, presence of said cytolytic T cell being indicative of said cancerous condition.

134. Method for treating a subject afflicted with a cancerous condition, comprising:

(i) removing a lymphocyte containing sample from said subject,

(ii) contacting the lymphocyte containing sample to a cell line transfected with a gene coding for and expressing a gene for a tumor rejection antigen precursor expressed by cancer cells associated with said conditions, under conditions favoring production of cytotoxic T cells against a tumor rejection antigen derived from said tumor rejection antigen precursor, and

(iii) introducing said cytotoxic T cells to said subject in an amount sufficient to lyse said cells.

135. Method for treating a subject afflicted with a cancerous condition, comprising:

(i) identifying a MAGE gene expressed by cancer cells associated with said condition;

(ii) identifying an HLA molecule which presents a portion of an expression product of said MAGE gene;

(iii) transfecting a host cell having the same HLA molecule as identified in (ii) with said MAGE gene;

(iv) culturing said transfected cells to express said MAGE-gene, and;

(v) introducing an amount of said cells to said subject sufficient to provoke an immune response against said tumor.

136. Method of claim 135, wherein said immune response comprises a B-cell response.

137. Method of claim 135, wherein said immune response is a T-cell response.

138. Method of claim 136, wherein said B cell response comprises production of antibodies specific to said tumor rejection antigen or tumor rejection antigen precursor.

139. Method of claim 137, wherein said T-cell response comprises generation of cytolytic T-cells specific for cells presenting said tumor rejection antigen.

140. Method of claim 139, further comprising treating said cells to render them non-proliferative.

141. Method for treating a subject with a cancerous condition, comprising:

(i) identifying a MAGE gene expressed by said tumor;

(ii) transfecting a host cell having the same HLA type as said patient with said MAGE gene;

(iii) culturing said transfected cells to express said MAGE gene, and;

(iv) introducing an amount of said cells to said subject sufficient to provoke an immune response against said tumor.

142. Method of claim 141, further comprising treating said cells to render them non proliferative.

143. Method for treating a subject with a cancerous condition, comprising administering to said subject an amount of a cell transfected with (i) a nucleic acid sequence which codes for a tumor rejection antigen precursor (TRAP) and (ii) a nucleic acid sequence which codes for an MHC or HLA molecule which presents a tumor rejection antigen derived from said TRAP, wherein said tumor rejection antigen is presented by cells associated with said cancerous condition, sufficient to alleviate said cancerous condition.

144. Method of claim 143, further comprising treating said cell to render it non-proliferative.

145. Method for preparing a biological material useful in treating a subject afflicted with a cancerous condition, comprising:

(i) transfecting a host cell with a nucleic acid molecule which codes for or expresses a tumor rejection antigen precursor;

(ii) transfecting said host cell with a nucleic acid molecule which codes for an HLA molecule which presents a tumor rejection antigen derived from said tumor rejection antigen precursor on a cell surface, and;

(iii) treating said host cells under conditions favoring expression of said nucleic acid molecules, and presentation of said tumor rejection antigen by said human leukocyte antigen.

146. Method of claim 145, further comprising treating said host cells to render them non proliferative following presentation of said tumor rejection antigen.

147. Method of claim 146, further comprising transfecting said host cell with a nucleic acid molecule which codes for or expresses a cytokine.

148. Method of claim 146, wherein said cytokine is an interleukin.

149. Method of claim 146, wherein said human leukocyte antigen is HLA-A1.

150. Method of claim 148, wherein said interleukin is IL-2.

151. Method of claim 146, wherein said interleukin is IL-4.

152. Method for treating a subject afflicted with a cancerous condition comprising administering to said subject an amount of a reagent consisting essentially of non-proliferative cell having expressed on its surface a tumor rejection antigen characteristic of cancerous cells in an amount sufficient to elicit an immune response thereto.

153. Method for treating a subject afflicted with a cancerous condition comprising administering to said subject an antibody which specifically binds to a tumor rejection antigen expressed on a cancer cell associated with said condition, said antibody being coupled to an anticancer agent, in an amount sufficient to treat said cancerous condition.

154. Method for treating a subject afflicted with a cancerous condition comprising administering to said subject an antibody which specifically binds to a

tumor rejection antigen precursor expressed by a cancer cell associated with said condition, said antibody being coupled to an anticancer agent, in an amount sufficient to treat said cancerous condition.

155. Method for treating a subject afflicted with a cancerous condition comprising administering to said subject a biological sample prepared in accordance with claim 142 in an amount sufficient to alleviate said cancerous condition.

156. Method for preventing onset of a cancerous condition in a subject comprising administering an amount of the vaccine of claim 77 in an amount sufficient to prevent onset of said cancerous condition in said subject.

157. Method for preventing onset of a cancerous condition in a subject comprising administering an amount of the vaccine of claim 78 in an amount sufficient to prevent onset of said cancerous condition in said subject.

158. Method for preventing onset of a cancerous condition in a subject comprising administering an amount of the vaccine of claim 82 in an amount sufficient to prevent onset of said cancerous condition in said subject.

159. Method for preventing onset of a cancerous condition in a subject comprising administering an amount of the vaccine of claim 86 in an amount sufficient to prevent onset of said cancerous condition in said subject.

160. Method for preventing onset of a cancerous condition in a subject comprising administering an amount of the vaccine of claim 87 in an amount sufficient to prevent onset of said cancerous condition in said subject.

161. Method for preventing onset of a cancerous condition in a subject comprising administering an amount of the vaccine of claim 88 in an amount sufficient to prevent onset of said cancerous condition in said subject.

162. Method for preventing onset of a cancerous condition in a subject comprising administering an amount of the vaccine of claim 89 in an amount sufficient to prevent onset of said cancerous condition in said subject.

163. Method for preventing onset of a cancerous condition in a subject comprising administering an amount of the vaccine of claim 89 in an amount sufficient to prevent onset of said cancerous condition in said subject.

164. Method for preventing onset of a cancerous condition in a subject comprising administering an amount of the vaccine of claim 90 in an amount sufficient to prevent onset of said cancerous condition in said subject.

165. Method for treating a subject afflicted with a cancerous condition, comprising:

(i) identifying cells from said subject which express a tumor rejection antigen precursor and present a tumor rejection antigen derived from said precursor on their surface;

(ii) isolating a sample of said cells;

(iii) cultivating said cell, and;

(iv) introducing said cells to said subject in an amount sufficient to provoke an immune response against said cells.

166. Method of claim 165, further comprising rendering said cells non proliferative, prior to introducing them to said subject.

167. Method for identifying a cytotoxic T cell useful in treating a subject afflicted with a cancerous condition, comprising:

(i) identifying a tumor rejection antigen presented by cells associated with said cancerous condition derived from a tumor rejection antigen

precursor expressed by said cells, prior to introducing them to said subject;

(ii) contacting a cell presenting said antigen to a cytotoxic T cell, and;

(iii) measuring a parameter selected from the group consisting of (i) proliferation of said cytotoxic T cell and (ii) release of a cytotoxic T cell produced factor, wherein increase in said parameter is indicative of said cancerous condition.

168. Method of claim 167, wherein said factor is tumor necrosis factor.

169. Method for following progress of a therapeutic regime designed to alleviate a cancerous condition, comprising:

(a) assaying a sample from a subject to determine level of a parameter selected from the group consisting of (i) tumor rejection antigen, (ii) a cytolytic T cell specific for cells presenting said tumor rejection antigen, and (iii) an antibody which specifically binds to said tumor rejection antigen at a first time period;

(b) assaying level of the parameter selected in (a) at a second period of time and comparing it to the level determined in (a) as a determination of effect of said therapeutic regime.

170. Method for diagnosing a cancerous condition comprising assaying a sample taken from a subject for expression of a TRAP molecule, and comparing levels of expression to a normal level, wherein variance there between is indicative of a cancerous condition.

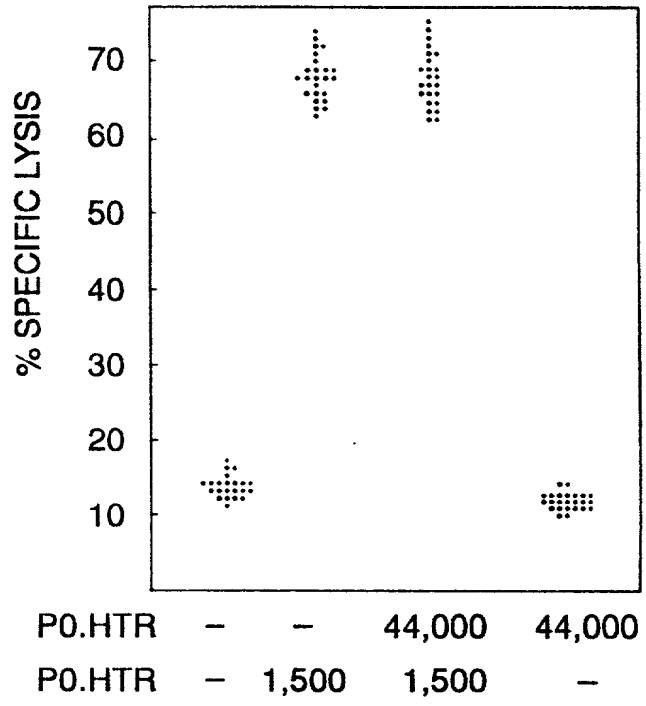
171. Method of claim 164, comprising measuring expression via polymerase chain reaction.

172. Method of claim 123, comprising intradermally administering an amount of a tumor rejection antigen sufficient to generate a delayed type response in a subject.

ABSTRACT OF THE DISCLOSURE

The invention relates to an isolated DNA sequence which codes for an antigen expressed by tumor cells which is recognized by cytotoxic T cells, leading to lysis of the tumor which expresses it. Also described are cells transfected by the DNA sequence, and various therapeutic and diagnostic uses arising out of the properties of the DNA and the antigen for which it codes.

FIG. 1A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50
40
30
20
10
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P0.HTR – 1,500 1,500 –

FIG. 1B

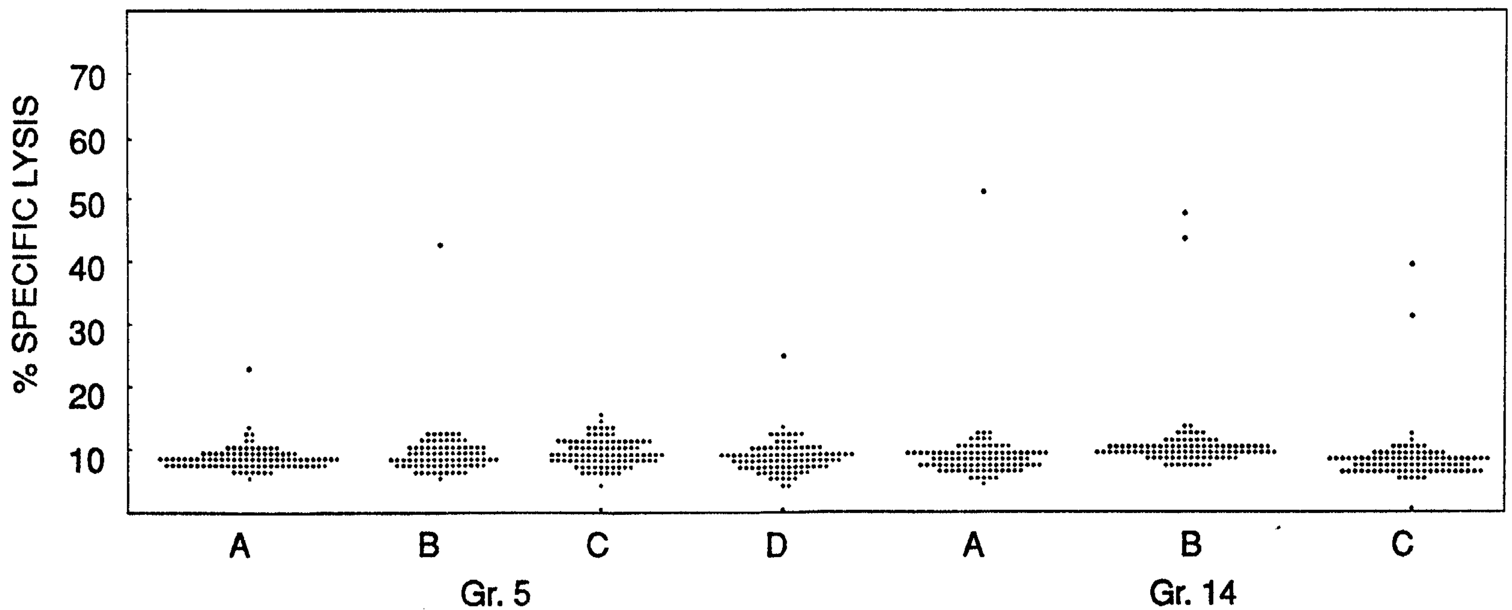
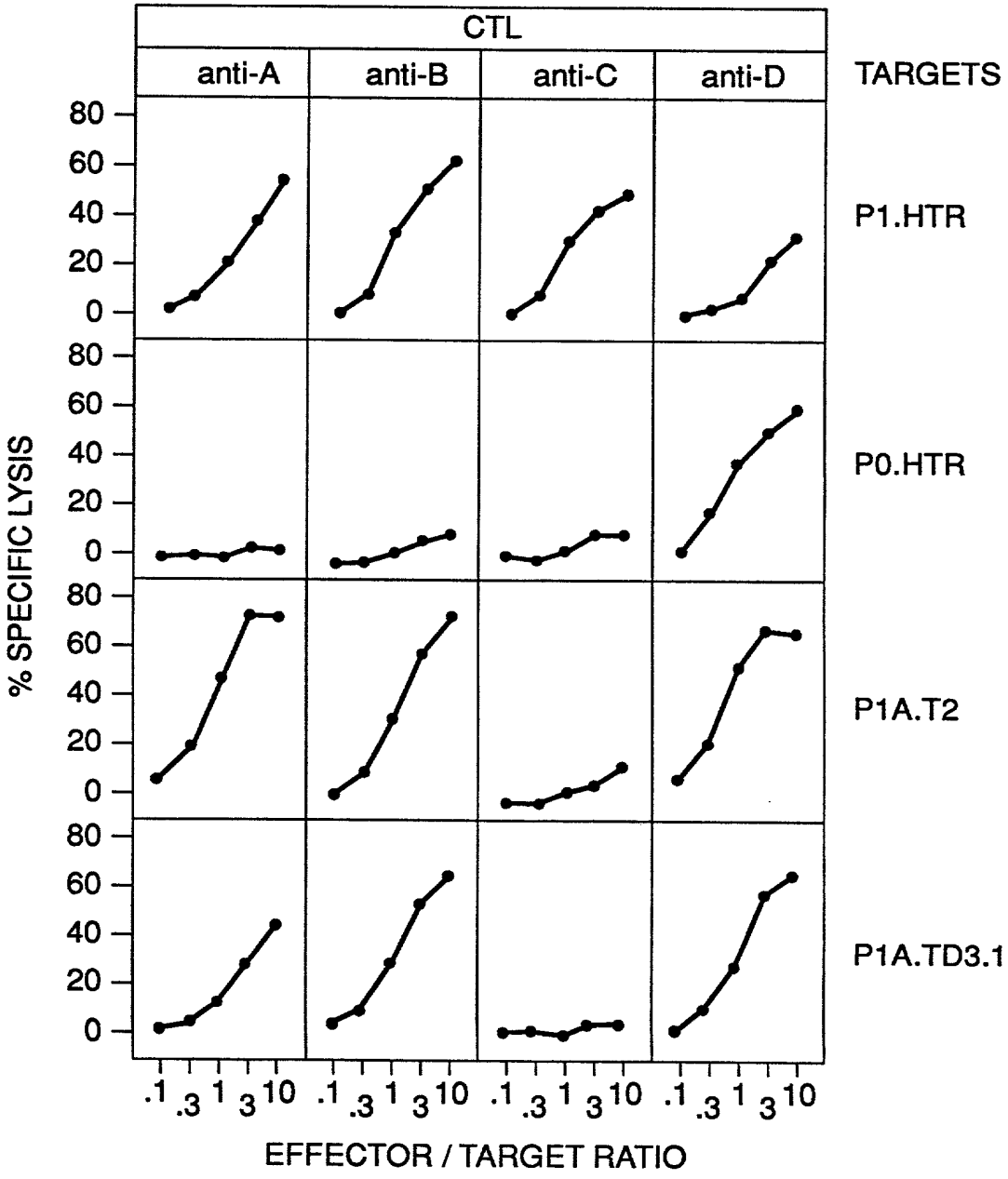


FIG. 2


CTL
anti-A
anti-B
anti-C
anti-D
TARGETS
P1.HTR
P0.HTR
P1A.T2
P1A.TD3.1
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20
40
60
80
.1
.3
1
3
10
EFFECTOR / TARGET RATIO

FIG. 3

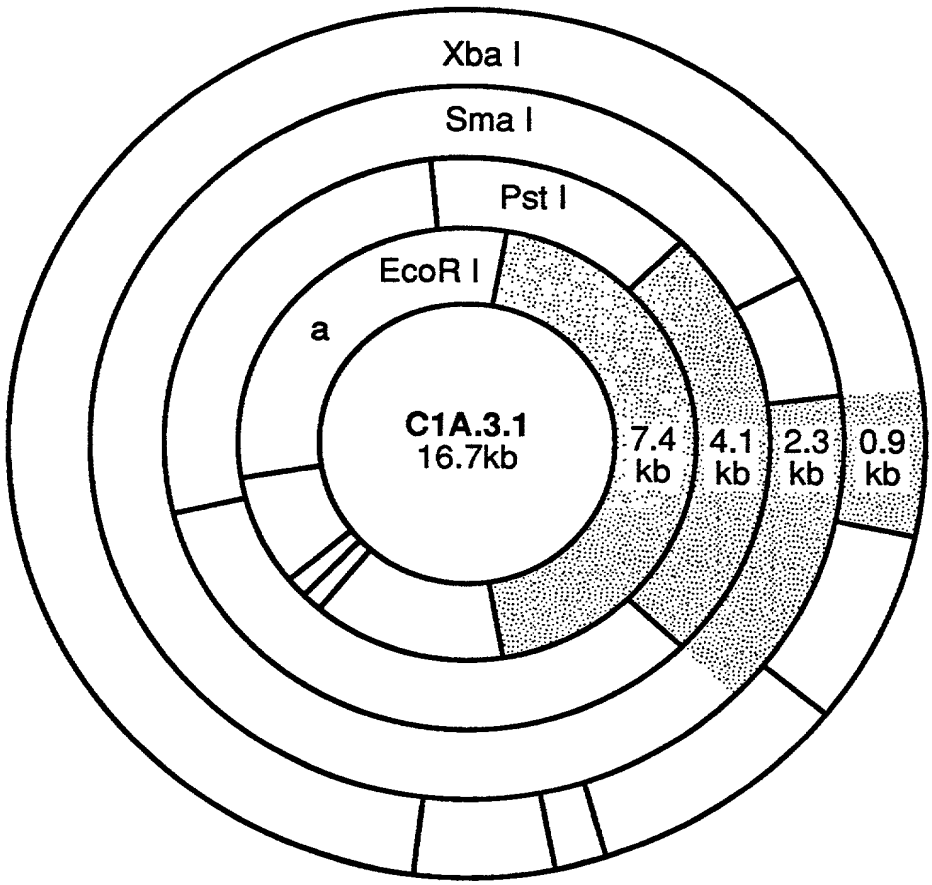


FIG. 4

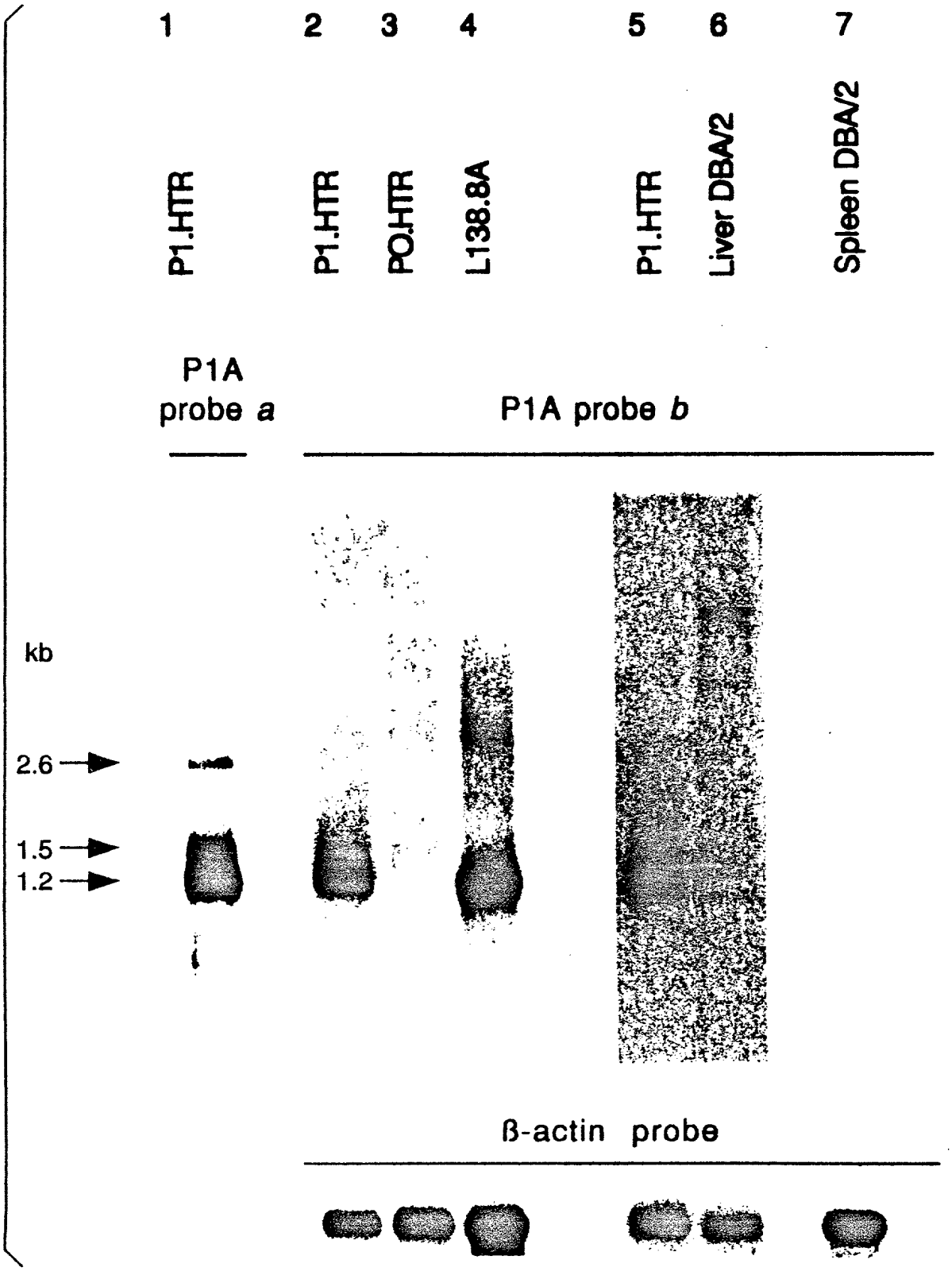

1
2
3
4
5
6
7
P1.HTR
P1.HTR
PO.HTR
L138.8A
P1.HTR
Liver DBA/2
Spleen DBA/2
P1A probe a
P1A probe b
kb
2.6
1.5
1.2
ß-actin probe

FIG. 5

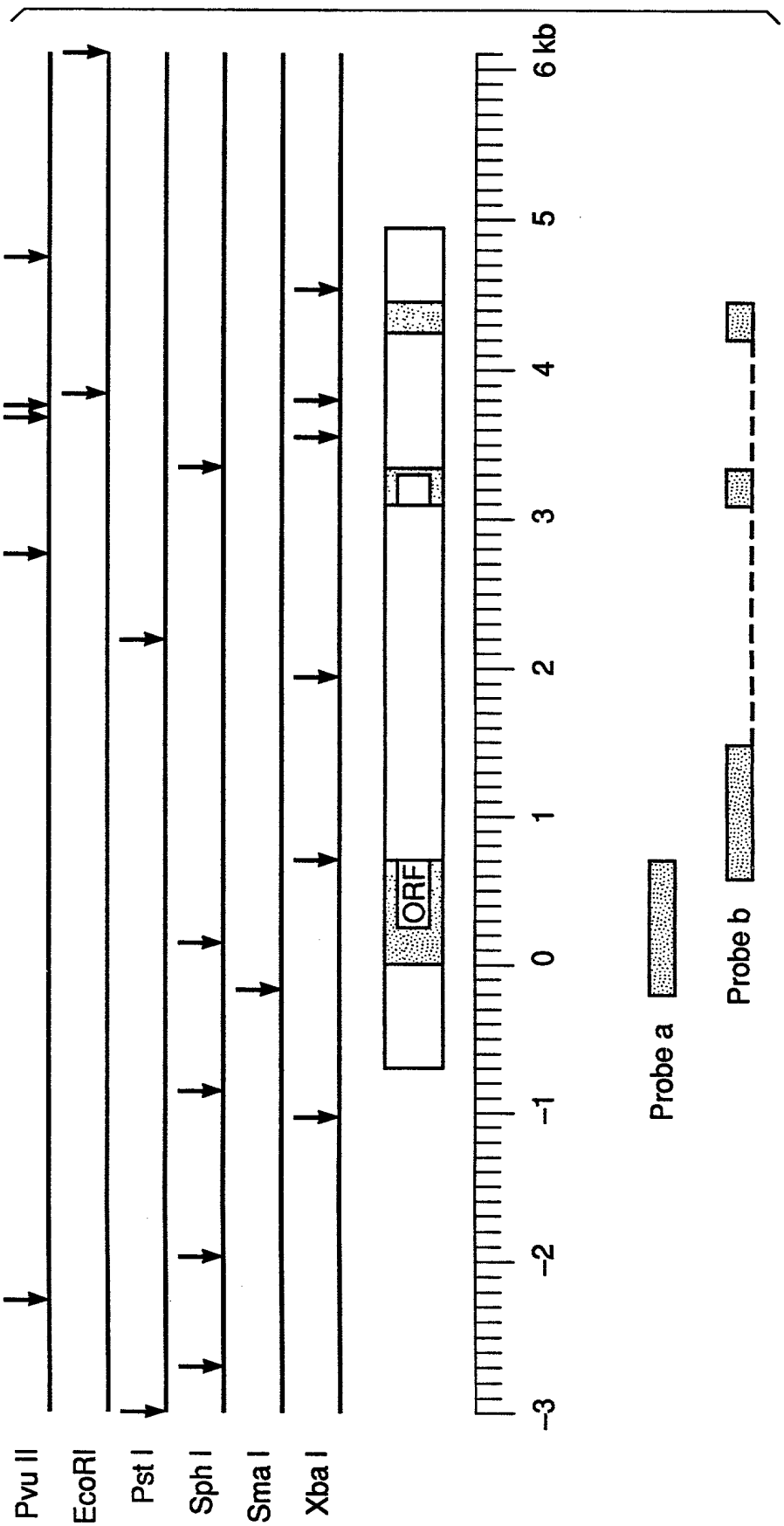


FIG. 6

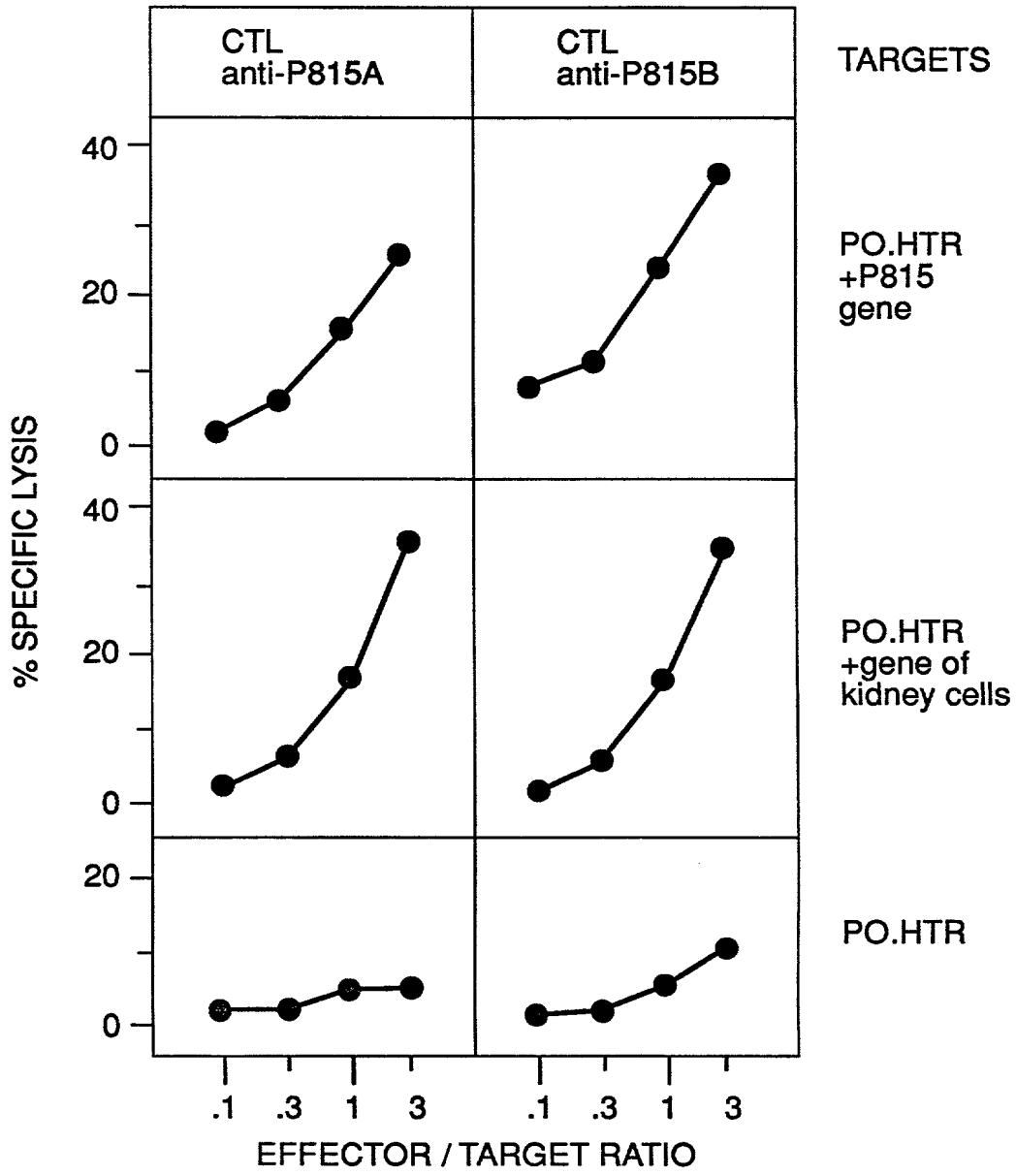
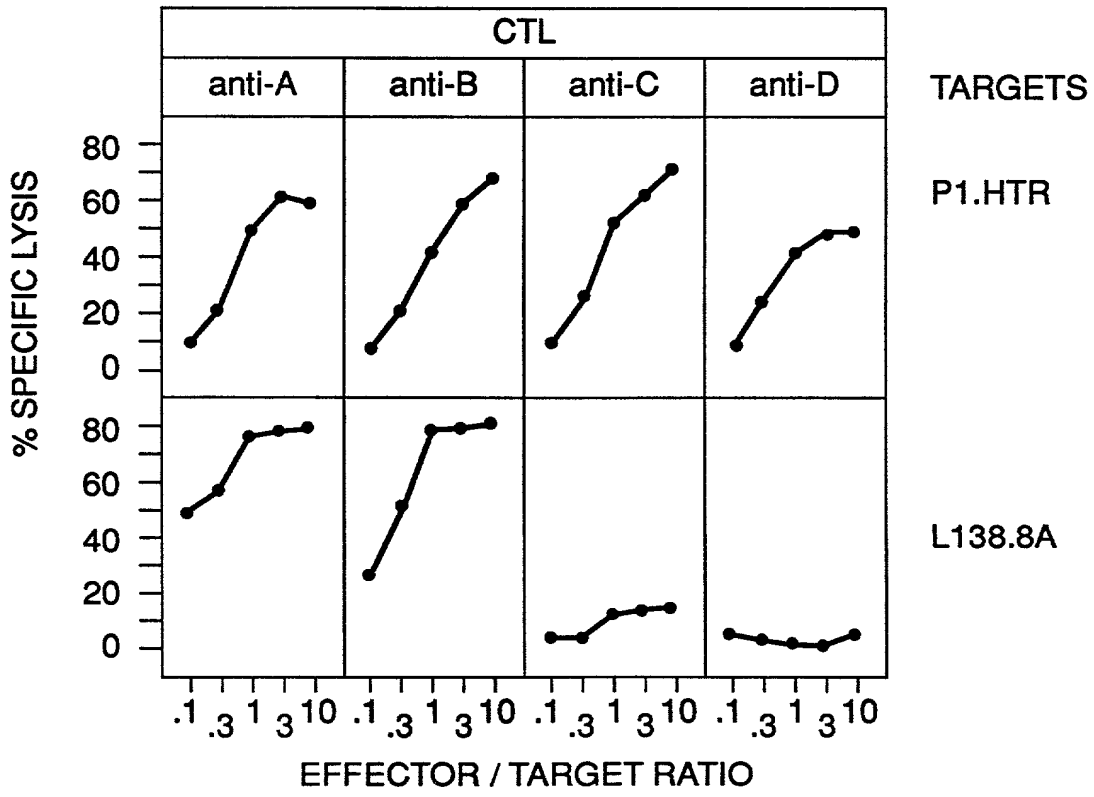

CTL
anti-P815A
CTL
anti-P815B
TARGETS
40
20
0
% SPECIFIC LYSIS
PO.HTR
+P815
gene
PO.HTR
+gene of
kidney cells
PO.HTR
.1
.3
1
3
EFFECTOR / TARGET RATIO

FIG. 7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anti-C
anti-D
TARGETS
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80
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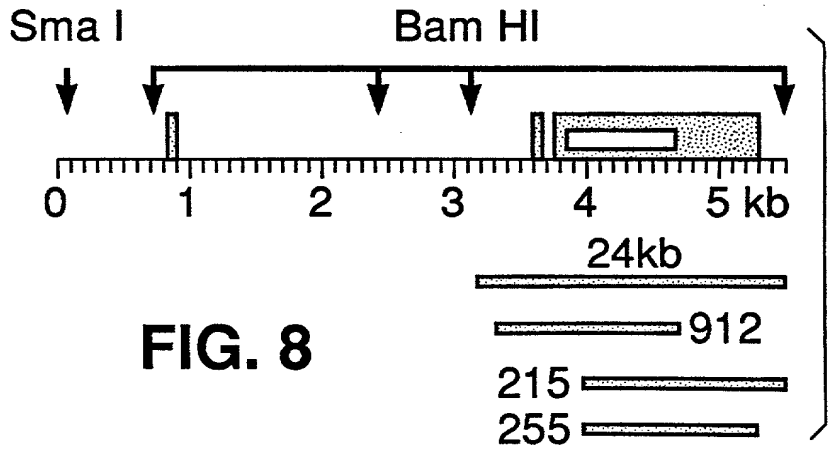
EFFECTOR / TARGET RATIO


Sma I
Bam HI
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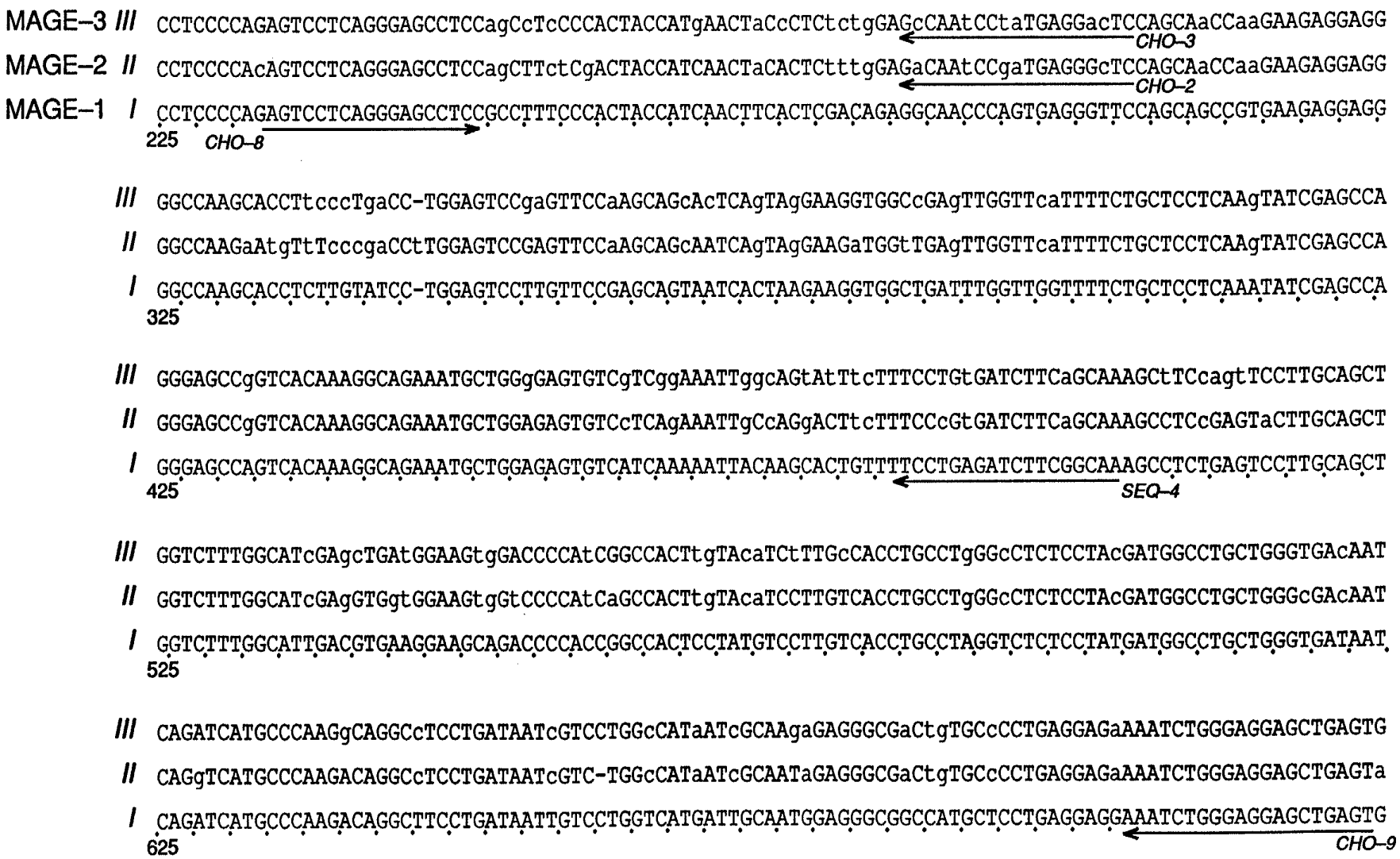
1
2
3
4
5 kb
24kb
912
215
255

FIG. 8

FIG. 9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CHO-3
MAGE-2 II CCTCCCCAcAGTCCTCAGGGAGCCTCCagCTTctCgACTACCATCAACTaCACTCtttgGAGaCAAtCCgaTGAGGGcTCCAGCAaCCaaGAAGAGGAGG
CHO-2
MAGE-1 I CCTCCCCAGAGTCCTCAGGGAGCCTCCGCCTTTCCCACTACCATCAACTTCACTCGACAGAGGCAACCCAGTGAGGGTTCCAGCAGCCGTGAAGAGGAGG
225
CHO-8
III GGCCAAGCACCTtcccTgaCC-TGGAGTCCgaGTTCCaAGCAGcAcTCAgTAgGAAGGTGGCcGAgTTGGTTcaTTTTCTGCTCCTCAAgTATCGAGCCA
II GGCCAAGaAtgTtTcccgaCCtTGGAGTCCGAGTTCCaAGCAGcAATCAgTAgGAAGaTGGtTGAgTTGGTTcaTTTTCTGCTCCTCAAgTATCGAGCCA
I GGCCAAGCACCTCTTGTATCC-TGGAGTCCTTGTTCCGAGCAGTAATCACTAAGAAGGTGGCTGATTTGGTTGGTTTTCTGCTCCTCAAATATCGAGCCA
325
III GGGAGCCgGTCACAAAGGCAGAAATGCTGGgGAGTGTCgTCggAAATTggcAGtAtTtcTTTCCTGtGATCTTCaGCAAAGCtTCcagtTCCTTGCAGCT
II GGGAGCCgGTCACAAAGGCAGAAATGCTGGAGAGTGTCcTCAgAAATTgCcAGgACTtcTTTCCcGtGATCTTCaGCAAAGCCTCcGAGTaCTTGCAGCT
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425
SEQ-4
III GGTCTTTGGCATcGAgcTGAtGGAAGtgGACCCCAtCGGCCACTtgTAcaTCtTTGcCACCTGCCTgGGcCTCTCCTAcGATGGCCTGCTGGGTGAcAAT
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525
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CHO-9

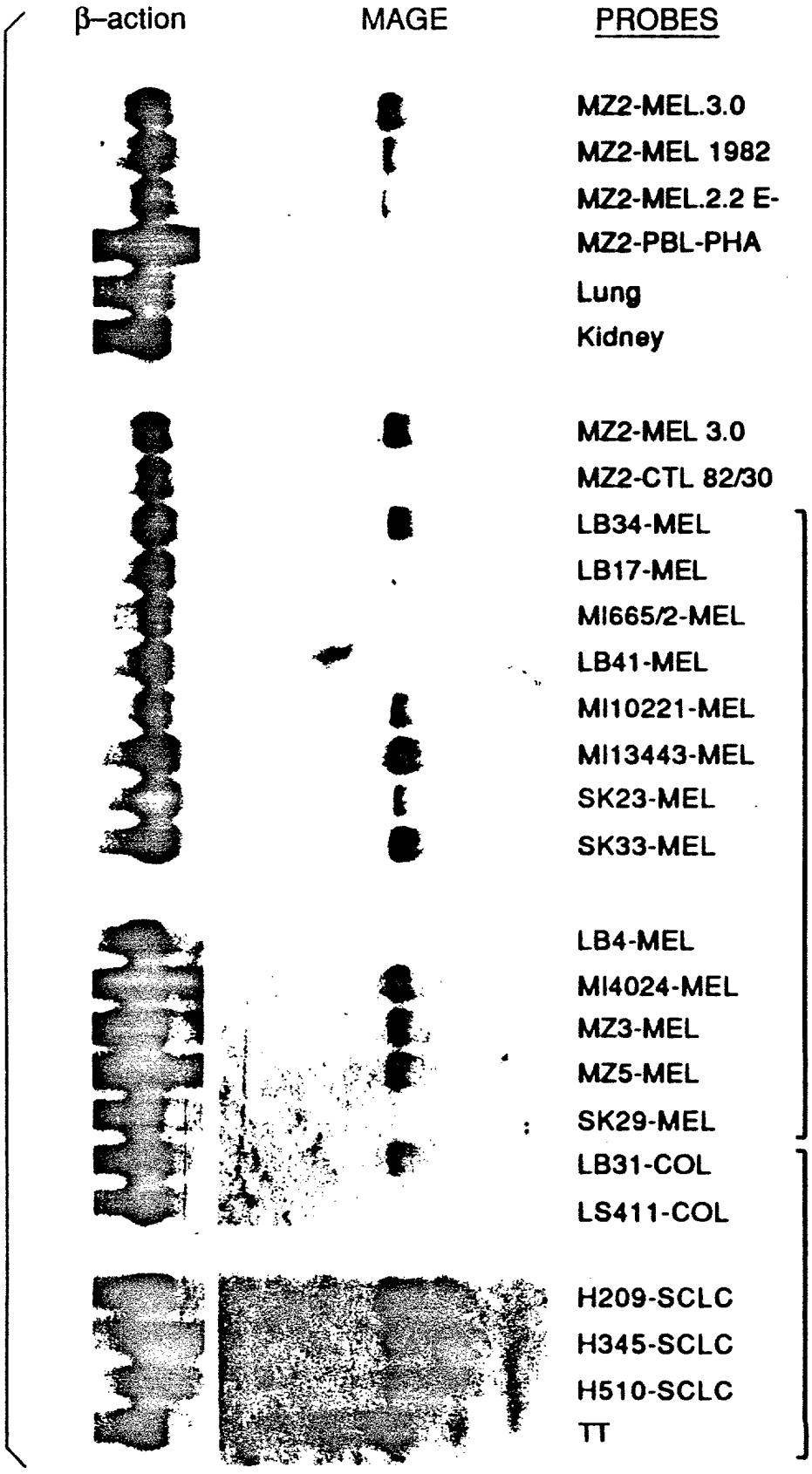


FIG. 10

FIG. 11

		EXPRSSION OF MAGE GENE FAMILY				RECOGNITIN BY ANI-E CTL		Expression of antigen MZ2-E after transaction**
		Northern blot probed with cross-reactive MAGE-1 probe*	cDNA-PCR product probed with oligonucleotide specific for:			tested by:		
			MAGE-1	MAGE-2	MAGE-3†	TNF release‡	Lysis§	
Cells of patient MZ2	melanoma cell line MZ2-MEL3.0	+	++++	+++++	+++++	+	+	
	tumor sample MZ2 (1982)	+	+++	+++	+++			
	antigen-loss variant MZ2-MEL2.2	+	–	+++	+++	–	–	
	CTL clone MZ2-CTL82/30	–	–	–	–			
	PHA-activated blood lymphocytes	–	–	–	–			
Normal tissues	Liver	–	–	–	–			
	Muscle	–	–	–	–			
	Skin	–	–	–	–			
	Lung	–	–	–	–			
	Brain	–	–	–	–			
	Kidney	–	–	–	–			
Melanoma cell lines of HLA-A1 patients	LB34-MEL	+	++	++++	++++	+	+–	
	MI665/2-MEL	–	–	–	–	–	–	+
	MI10221-MEL	+	–	++	+++	–	–	+
	MI13443-MEL	+	+++	++++	++++	+	+	
	SK33-MEL	+	–	++++	++++	–	–	–
	SK23-MEL	+	–	++++	++++	–	–	+
Melanoma cell lines of other patients	LB17-MEL	+	+	++++	++++	–	–	–
	LB33-MEL	+	–	+++	+++	–	–	–
	LB4-MEL	–	–	–	–	–	–	
	LB41-MEL	–	–	–	–	–	–	
	MI4024-MEL	+	+++	++++	++++	–	–	
	SK29-MEL	–	–	–	–	–	–	
	MZ3-MEL	+	+	++++	++++	–	–	
	MZ5-MEL	+	–	++++	++++	–	–	
Melanoma tumor sample	BB5-MEL	+	+++	++	+++			
Other tumor cell lines	small cell lung cancer H209	+	–	++++	++++			
	small cell lung cancer H345	+	–	++++	++++			
	small cell lung cancer H510	+	–	++++	++++			
	small cell lung cancer LB11	+	+	++++	++++			
	bronchial squamous cell carcinoma LB37	+	–	–	+++			
	thyroid medullary carcinoma TT	+	++++	+++	++++			
	colon carcinoma LB31	+	–	+++	++++	–		
	colon carcinoma LS411	–	–	–	–			
Other tumor samples	chronic myeloid leukemia LLC5	–	–	–	–			
	acute myeloid leukemia TA	–	–	–	–			

* Data obtained in the conditions of figure 5.
† Data obtained as described in figure 6.
‡ TNF release by CTL 82/30 after stimulation with the tumor cells as described in (11).
§ Lysis of 51 Cr labelled target by CTL 82/30 in the conditions of figure 1.
** Cells transfected with the 2.4 kb fragment of gene MAGE-1 were tested for their ability fo stimulate TNF release by CTL 82/30

FIG. 12

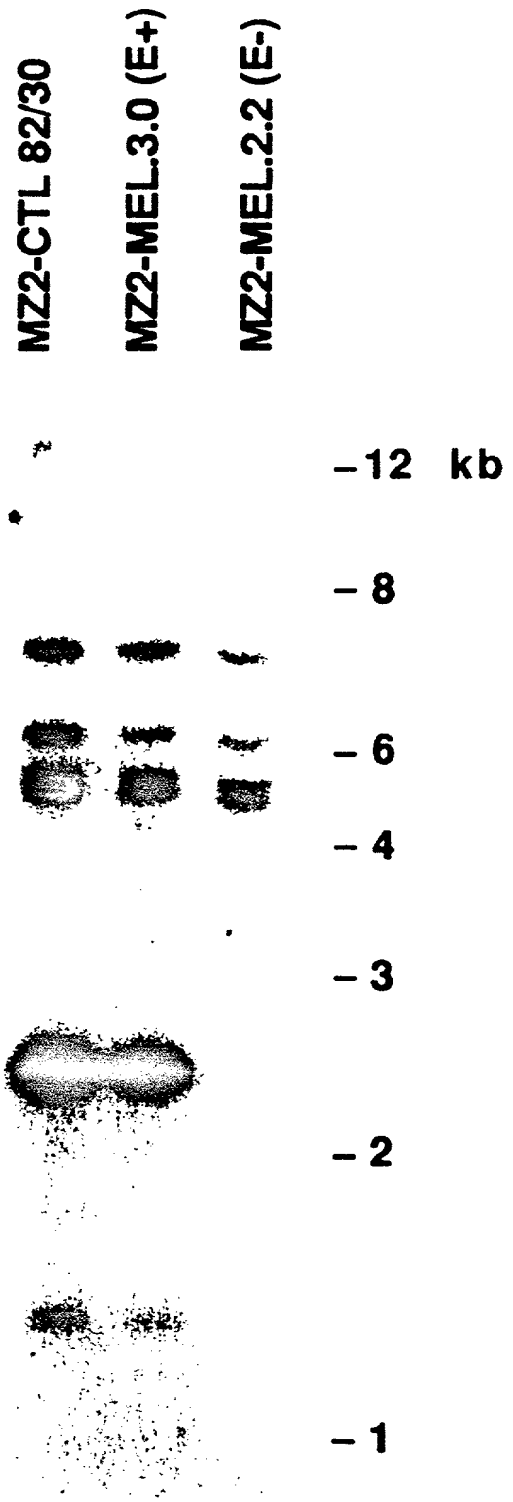
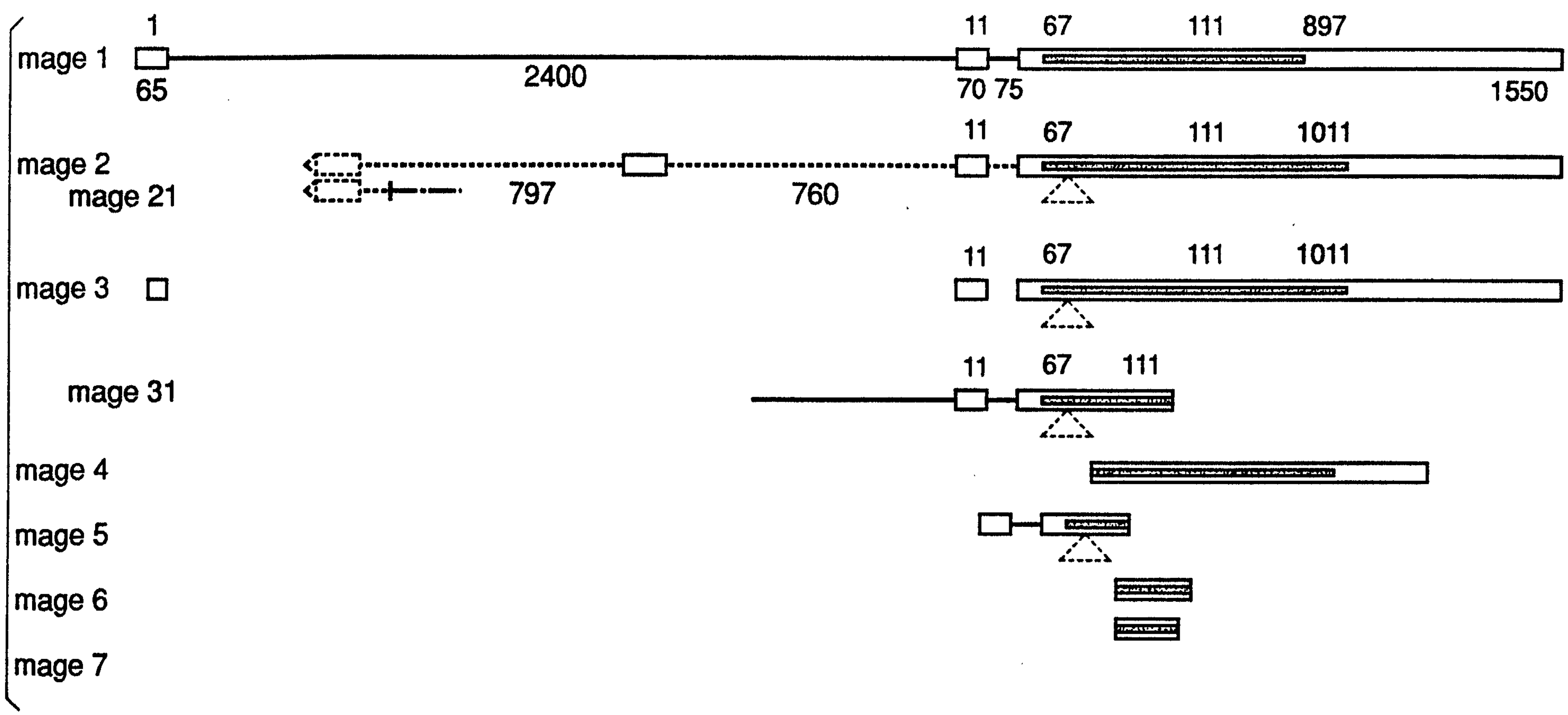


FIG. 13


1
11
67
111
897
mage 1
65
2400
70 75
1550
11
67
111
1011
mage 2
mage 21
797
760
11
67
111
1011
mage 3
11
67
111
mage 31
mage 4
mage 5
mage 6
mage 7